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B-Cell Activation and Tolerance Mediated by B-Cell Receptor, Toll-Like Receptor, and Survival Signal Crosstalk in SLE Pathogenesis

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CONTRACTING ORGANIZATION:

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13. SUPPLEMENTARY NOTES					
14. ABSTRACT We previously found that B cell receptor (BCR)-delivered TLR9 agonists initiate a response involving proliferation followed by abrupt cell death; furthermore, responding cells are rescued by survival cytokines. We posited this as a normal immune response-limiting mechanism that, if thwarted, may lead to persistence of self-reactive antibody-secreting cells. In this proposal we seek to characterize the pathways leading to post-proliferative death and rescue, and to determine how different forms of rescue lead to alternative differentiation outcomes. During the first year period we showed that in the context of BCR-delivered TLR9 signals, IL-21 promotes and IL-4 opposes the T-bet+CD11c+ B cell fate. In the current reporting period, we have extended these findings to show that IFN-gamma also promotes the Tbet+ fate, and that B cells with this phenotype are antigen-experienced cells that emerge in both normal responses to viral infections as well as in autoimmune scenarios. We have forwarded a theoretical framework to explain the link between these activation requisites and humoral autoimmunity.					
15. SUBJECT TERMS regulation of B cell responses; TLR7/9 agonists					
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1. INTRODUCTION: This grant is based on our observation that BCR-delivered TLR9 agonists initiate a self-limiting response involving proliferation and differentiation, followed by abrupt cell death, but that B cells can be rescued from death and directed toward effector fates by survival and differentiation mediators. The planned and ongoing studies are thus to investigate the outcomes of this rescue and differentiation in both murine and human cells – with emphasis on how different forms of rescue lead to alternative fates associated with autoimmune disease.

2. KEYWORDS: Tbet-postive B cell, ABC, autoimmunity, SLE

3. ACCOMPLISHMENTS:

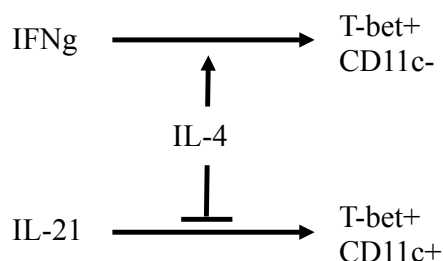
Major goals as stated in the approved SOW: Specific Aim 1: Detail the pathways mediating post proliferative death, rescue, and differentiation of each peripheral B cell subset in mice and humans. Major Task 1.1: Determine characteristics of death and rescue among several additional mouse and human B cell subsets. Major Task 1.2: Assess the intracellular pathways that mediate cell death and rescue in each B cell subset following BCR-delivered TLR9 agonists. Major Task 1.3: Further characterize the signaling systems involved in post proliferative death and rescue).

Specific Aim 2: Assess how alternative forms of rescue mediate distinct differentiative outcomes. Major Task 2.1: Assess whether alternative exogenous signals can rescue post-proliferative cell death. Major Task 2.2: Determine the signals through which BLyS versus alternative mechanisms rescue cells following BCR-delivered TLR9 agonists. Major Task 2.3: Establish whether cells of each B cell subset adopt plasma cell or germinal center programs following different forms of rescue.

Specific Aim 3: Determine whether B cells from selected SLE patients are refractory to post-proliferative death mediated by TLR9 agonists, or more responsive to plasmablast or germinal center B cell differentiation in response to rescue signals. Major Task 3.1: Assess the response of B cells from SLE patients with potential defects in TLR9 signaling. Major Task 3.2: Determine whether B cells from individuals or mouse lines harboring a risk allele are refractory to post-proliferative death mediated by TLR9 agonists.

What were the major goals of the project? In summary, the goals of the project are to detail the pathways mediating post-proliferative death, rescue, and differentiation of each peripheral B cell subset in mice and humans, and to assess how alternative forms of rescue mediate distinct differentiative outcomes.

What was accomplished under these goals? Our progress toward these goals during the current year has culminated in one primary research publication, one interpretive review article, and a second primary research publication under review. This work details mechanisms of the self-limiting response (Major Tasks 1.1 through 1.3 and 3.2), mechanisms of rescue (Major Tasks 2.1 and 2.2), and consequences of rescue in terms of cell fate (Major Task 2.3). Briefly, BCR-delivered TLR9 ligands induce cell cycle arrest and mitochondrial apoptosis; this is characteristic of all primary mouse and human B cell subsets, including the mouse transitional B cell subset which is known to contain a high proportion of autoreactive B cells. Rescue is achieved with BLyS (BAFF), CD40 costimulation, or CD40 plus TFH cytokines. IFN γ or IL-21 promotes Tbet expression in the context of TLR9 engagement; IL-4 blocks this effect; and IL-21 but not IFN γ promotes expression of CD11c (integrin α X). These general attributes are summarized in the diagram at left. Further, we have shown that Tbet⁺ B cells arise during responses to some viral and helminth infections, indicating that they are a B cell subset that occurs normally under certain circumstances. Taken together, our results suggest that concurrent BCR, TLR9, and cytokine signals regulate adoption of the Tbet⁺ B cell phenotype, which can mediate either a protective response (e.g. to a pathogen-associated nucleic acid) or a pathogenic response (e.g. to self-antigens such as chromatin or apoptotic debris). These observations are now published (Naradikian et al., J Immunol. 2016 Aug 15;197(4):1023-8. doi: 10.4049/jimmunol.1600522. Epub 2016 Jul 18, copy appended to this report).



In separate work conducted collaboratively with the Gearhart Lab, submitted to the Journal of Immunology and under revision, we show that ABCs are likely an antigen experienced population that results during T-dependent B cell responses, inasmuch as they require CD40/154 interactions and B cell intrinsic MHC class II expression for their accumulation (Russel Knode et al.).

In work submitted to the Journal of Clinical Investigation and under revision, (Sindhava et al), we show that DNA immune complexes (BCR-delivered TLR9 ligand) induce a self-limiting B cell response: a burst of proliferation followed by death. The mechanism involves p38 MAPK-mediated cell cycle arrest followed by mitochondrial apoptosis. All pre-immune murine B cell subsets undergo TLR9-dependent post-proliferative death, and cells can be rescued to antibody secretion by BLyS (BAFF). Human CD27- B cells also undergo proliferation-associated apoptosis following stimulation with BCR-delivered TLR9 ligand. CD40 costimulation alone rescues, and CD40 costimulation in the presence of IL-21 or IFN γ leads to Tbet upregulation and class switching to IgG2c. Using a previously reported method to produce amyloid protein antigens linked to another protein (human serum albumin) (AP-HSA), AP-HSA linked to DNA, or HSA mixed with but not linked to DNA, we show that the antigen-complexed DNA leads to blunted antibody responses and switching to IgG2c (Figure 1).

Fig 4

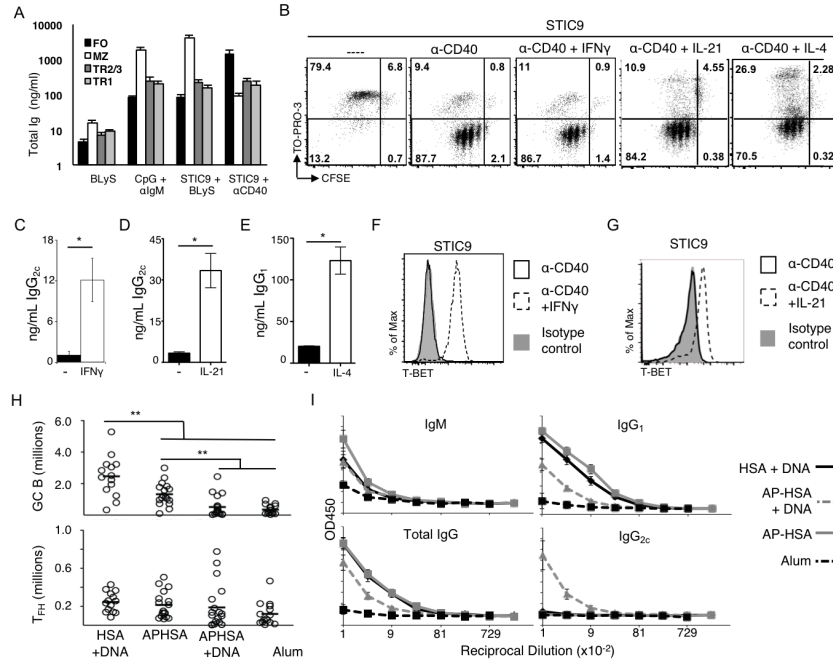


Figure 1 (Fig. 4 of manuscript under revision): CD40 and TFH cytokines promote survival, T-bet expression and IgG2c class switching in STIC9 stimulated cells, and DNA conjugation modulates T-dependent B cell responses *in vivo*. (A) Sort-purified FO, MZ, TR2/3, and TR1 B cells cultured for 60hrs with ODN 1826 + F(ab)₂ fragments of anti-IgM, STIC9, STIC9 + BLYS, or STIC9+anti-CD40. At the end of the culture, total Ig in the supernatants was measured by ELISA. (B) FACS analysis of proliferation and survival of Fo B cells cultured for 60hrs with STIC9, STIC9 + anti-CD40, STIC9 + anti-CD40 + IFN γ , STIC9 + anti-CD40 + IL21, or STIC9 + anti-CD40 + IL-4. Dead cells were stained by TO-PRO-3, while CFSE dilution indicates proliferation. (C, D, E) Fo B cells were cultured with STIC9 + anti-CD40 with or without (C) IFN γ , (D) IL-21, or (E) IL-4 for 60hrs. At the end of the culture, (C, D) IgG2c and (E) IgG1 were measured by ELISA. (F, G) Fo B cells were cultured with STIC9 + anti-CD40 with or without (F) IFN γ , or (G) IL-21 for 60hrs. At the end of the culture cells were probed for T-bet. Plots show T-bet expression in live cells. (H) Total number of splenic GC B cells and T_{FH} cells present in the indicated immunized mice at day 14 post-immunization. Each symbol represents individual mouse. (I) HSA-specific serum titer in the indicated immunized mice at day 14 post-immunization. For all data, n \geq 3 and representative of minimum of 3 independent experiments. * P < 0.05, ** P < 0.01.

In work conducted collaboratively with the Ettinger laboratory, we have established that IL-21 regulates CD11c expression by naïve human B cells, and likely contributes to autoimmunity through generation of self-reactive CD11c+ B cells in SLE (Casey et al., submitted).

The importance and interpretation of these findings is discussed in Naradikian et al., Immunol Rev. 2016 Jan;269(1):118-29. doi: 10.1111/imr.12380. PMID: 26683149, copy appended to this report). In summary, we propose that Tbet+ B cells are a memory B cell subset generated by BCR-delivered TLR9 or TLR7 agonists in the context of an inflammatory cytokine milieu (Figure 2 of this report).

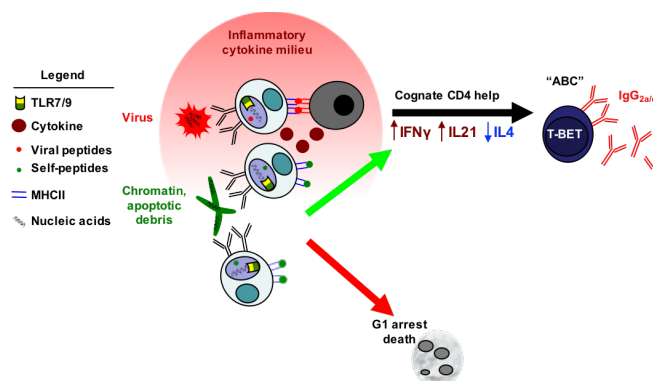


Figure 2. Beneficial and pathogenic ABCs arise via a common triad of signals. Preimmune FO, MZ, and/or TR B cells bind, internalize and traffic antigens to endocytic compartments via the BCR. Pathogen degradation leads to viral nucleic acids engaging TLR7 or TLR9. Processing and loading of viral peptides onto MHCII molecules results in cognate CD4 T cell interactions and T cell derived inflammatory cytokine secretion. The combination of these signals leads to T-BET⁺ memory and effectors, and to IgG_{2a/c} isotype switching. Normally, B cells that internalize nucleic acid containing self-antigens fail to survive because they lack the cognate T cell interactions and appropriate cytokine signals to further differentiate and survive. However, inadvertent or aberrant receipt of these signals can afford survival of these self reactive cell and enable their recruitment into a long lived memory and effector ABC pool. (adapted from Naradikian et al, Immunol. Rev. 2016, appended)

As progress towards elucidating the molecular pathways that engender beneficial Tbet+ B cells, and possibly protect against generation of self-reactive memory B cells (Major Tasks 1.3 and 2.1), we are performing both phosphoproteomic and metabolic studies on these populations. Phosphoproteomic studies will allow us to compare the phosphorylation status of hundreds of proteins in various treatment groups – for example, cell cultures treated with STIC9 (BCR-delivered TLR9 ligand), cultures treated with anti-mu plus CpG, and untreated cultures.

What opportunities for training and professional development has the project provided? Although training is not a goal of the project per se, during Year 2 these studies helped to serve as a research training vehicle for a graduate trainee (M. Naradikian) who defended his PhD research thesis in May 2016 and is now pursuing postdoctoral studies at Novartis, San Francisco. His primary support was from a USPHS T32 grant but he received a small portion of his graduate stipend from this project. In addition, two postdoctoral trainees have been partially supported by this project, Drs. Lauren E. Higdon and Arpita Myles. Dr. Higdon has gone on to her second post doc and Dr. Myles remains partially supported by this grant. Finally, a former postdoc with related research experience, Vishal J. Sindhava, returned to the lab as a Research Associate and is partially supported by this project.

How were the results disseminated to communities of interest? The work under this award has contributed to several research papers or reviews/commentaries in peer reviewed journals (see below). In addition, some aspects of the work were presented at the 2016 Midwinter Immunology Conference (talk by MP Cancro), the 2016 American Association of Immunologists meeting (talks by VJ Sindhava and A Myles), and the 2016 Keystone B Cell meeting (talk presented by MP Cancro and a poster presented by R Ettinger with MP Cancro contributing author). In addition, Dr. Cancro has presented aspects of the work during invited seminars or plenary talks at academic and research institutions, including Univ. of British Columbia, MedImmune, LLC, University of Erlangen, and University of Miami.

What do you plan to do during the next reporting period to accomplish the goals?

Ongoing studies will extend these findings to B cells in SLE patients (Specific Aim 3). We will further interrogate the downstream intracellular pathways that mediate rescue and cell fate adoption, as well as the phosphoproteomic and metabolomic profiles characterizing these cells.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Progress to date has been strong, and has uncovered an important association between certain forms of rescue – likely those mediated by TFH cytokines – with adoption of a cell fate that is associated with humoral autoimmunity. This may lead to an understanding of the origin of B cells responsible for producing detrimental antibodies in autoimmune diseases, particularly SLE and related diseases.

What was the impact on other disciplines? Nothing to report.

What was the impact on technology transfer? Nothing to report.

What was the impact on society beyond science and technology? Nothing to report.

5. CHANGES/PROBLEMS: Nothing to report.

6. PRODUCTS

PUBLICATIONS: The following publications resulted in full or in part from this grant support.

1. Sharma S, Fitzgerald KA, **Cancro MP**, Marshak-Rothstein A. Nucleic Acid-Sensing Receptors: Rheostats of Autoimmunity and Autoinflammation. *J Immunol.* 2015 Oct 15;195(8):3507-12. doi: 10.4049/jimmunol.1500964. Review. PubMed PMID: 26432899; PubMed Central PMCID: PMC4593056.
2. Rubtsova K, Rubtsov AV, **Cancro MP**, Marrack P. Age-Associated B Cells: A T-bet-Dependent Effector with Roles in Protective and Pathogenic Immunity. *J Immunol.* 2015 Sep 1;195(5):1933-7. doi: 10.4049/jimmunol.1501209. Review. PubMed PMID: 26297793; PubMed Central PMCID: PMC4548292.
3. Nündel K, Green NM, Shaffer AL, Moody KL, Busto P, Eilat D, Miyake K, Oropallo MA, **Cancro MP**, Marshak-Rothstein A. Cell-intrinsic expression of TLR9 in autoreactive B cells constrains BCR/TLR7-dependent responses. *J Immunol.* 2015 Mar 15;194(6):2504-12. doi: 10.4049/jimmunol.1402425. Epub 2015 Feb 13. PubMed PMID: 25681333; PubMed Central PMCID: PMC4382804.
4. Naradikian MS, Myles A, Beiting DP, Roberts KJ, Dawson L, Herati RS, Bengsch B, Linderman SL, Stelekati E, Spolski R, Wherry EJ, Hunter C, Hensley SE, Leonard WJ, **Cancro MP**. Cutting Edge: IL-4, IL-21, and IFN- γ Interact to Govern T-bet and CD11c Expression in TLR-Activated B cells. *J Immunol.* 2016 Aug 15;197(4):1023-8. doi: 10.4049/jimmunol.1600522. Epub 2016 Jul 18. PMID: 27430719 **APPENDED**
5. Naradikian MS, Hao Y, **Cancro MP**. Age Associated B cells: Key mediators of both protective and autoreactive humoral responses. *Immunol Rev.* 2016 Jan;269(1):118-29. doi: 10.1111/imr.12380. PMID: 26683149 **APPENDED**

Three additional manuscripts based on the findings described above are also currently under revision or review.

6. Sindhava VJ, Oropallo MA, Moody K, Naradikian MS, Higdon LE, Zhou L, Myles A, Green N, Nündel K, Stohl W, Schmidt AM, Cao W, Dorta-Estremera S, Kambayashi T, Marshak-Rothstein A, **Cancro MP**. A TLR9-dependent mechanism terminates B cell responses to DNA containing antigens, but is reversed by cytokine signals to yield antibody secretion and T-bet⁺ B cell differentiation. **UNDER REVIEW**, *J Clin Invest.*
7. Russel Knode LM, Naradikian MS, Scholz JL, Hao Y, Liu D, Ford ML, Tobias JW, **Cancro MP**, Gearhart PJ. Age-associated B cells express a diverse repertoire of mutated immunoglobulins and share transcriptional profiles with memory cells. **UNDER REVISION**, *J Immunol.*
8. Wang S, Want J, Naiman B, Karnell J, Gross P, Rahman S., Siegel R, Hasni S, **Cancro MP**, Kolbeck R, Ettinger R. CD11c expression in T-bet⁺ B cells is driven by IL-21 and associated with autoimmune disease manifestations in SLE. **UNDER REVISION**

Presentations:

Midwinter Immunology Conference,
American Association of Immunologists Annual Meeting,
Keystone B Cell Symposium (all in the first half of 2016)
University of British Columbia/Fraser University, Vancouver, Canada
University of Miami

Website(s) or other Internet site(s) Nothing to report.

Technologies or techniques Nothing to report.

Inventions, patent applications, and/or licenses Nothing to report.

Other Products Nothing to report.

7. PARTICIPANTS AND COLLABORATING ORGANIZATIONS:

Name	Michael P. Cancro
Project Role	P.I.
Researcher Identifier	N/A
Nearest person month worked	2
Contribution to project	Principal Investigator; oversee all research
Funding support	This award; R01-AI-118691 (NIAID)

Name	Jean L. Scholz
Project Role	Research Associate
Researcher Identifier	N/A
Nearest person month worked	3
Contribution to project	Perform experiments and oversee logistics
Funding support	This award; R01-AI-118691 (NIAID)

Name	Martin Naradikian
Project Role	Graduate student
Researcher Identifier	N/A
Nearest person month worked	6
Contribution to project	Perform experiments related to Tbet+ B cells
Funding support	This award; R01-AI-118691 (NIAID); NIH training grant.

Name	Arpita Myles
Project Role	Postdoc
Researcher Identifier	N/A
Nearest person month worked	3
Contribution to project	Perform experiments related to Tbet+ B cells, cell signaling mechanisms
Funding support	This award; R01-AI-118691 (NIAID)

Name	Vishal J. Sindhava
Project Role	Research Associate
Researcher Identifier	N/A
Nearest person month worked	2
Contribution to project	Perform experiments related to Tbet+ B cells, cell cycle / cell signaling
Funding support	This award; R01-AI-118691 (NIAID)

Name	Kenneth J. Roberts
Project Role	Technician
Researcher Identifier	N/A
Nearest person month worked	6
Contribution to project	Prepare reagents, run assays, organize animal colony
Funding support	This award; R01-AI-118691 (NIAID)

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

No change.

The following grants were received or renewed during the past year:

R01 AI118691-01; Cancro, Michael (PI); 02/10/15-01/31/20

Mechanistic studies of BLyS-mediated modulation in HIV-1 Env-specific antibody responses

T32 AI055428-12; Cancro, Michael (PI); 06/01/03-07/31/19

Training Program in Immune System Development and Regulation

What other organizations were involved as partners?

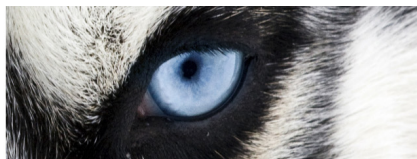
Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS: Does not apply.

9. APPENDICES:

Appendix 1: Naradikian MS, Myles A, Beiting DP, Roberts KJ, Dawson L, Herati RS, Bengsch B, Linderman SL, Stelekati E, Spolski R, Wherry EJ, Hunter C, Hensley SE, Leonard WJ, **Cancro MP**. Cutting Edge: IL-4, IL-21, and IFN- γ Interact to Govern T-bet and CD11c Expression in TLR-Activated B cells. J Immunol. 2016 Aug 15;197(4):1023-8. doi: 10.4049/jimmunol.1600522. Epub 2016 Jul 18. PMID: 27430719

Appendix 2: Naradikian MS, Hao Y, **Cancro MP**. Age Associated B cells: Key mediators of both protective and autoreactive humoral responses. Immunol Rev. 2016 Jan;269(1):118-29. doi: 10.1111/imr.12380. PMID: 26683149



Cutting Edge: IL-4, IL-21, and IFN- γ Interact To Govern T-bet and CD11c Expression in TLR-Activated B Cells

This information is current as
of July 28, 2016.

Martin S. Naradikian, Arpita Myles, Daniel P. Beiting,
Kenneth J. Roberts, Lucas Dawson, Ramin Sedaghat Herati,
Bertram Bengsch, Susanne L. Linderman, Erietta Stelekati,
Rosanne Spolski, E. John Wherry, Christopher Hunter, Scott
E. Hensley, Warren J. Leonard and Michael P. Cancro

J Immunol published online 18 July 2016
<http://www.jimmunol.org/content/early/2016/07/16/jimmunol.1600522>

Supplementary Material	http://www.jimmunol.org/content/suppl/2016/07/16/jimmunol.1600522.DCSupplemental.html
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Cutting Edge: IL-4, IL-21, and IFN- γ Interact To Govern T-bet and CD11c Expression in TLR-Activated B Cells

Martin S. Naradikian,^{*,†} Arpita Myles,^{*,†} Daniel P. Beiting,^{†,‡} Kenneth J. Roberts,^{*} Lucas Dawson,^{†,‡} Ramin Sedaghat Herati,^{†,§} Bertram Bengsch,^{†,¶} Susanne L. Linderman,^{†,¶,||} Erietta Stelekati,^{†,¶} Rosanne Spolski,[#] E. John Wherry,^{†,¶} Christopher Hunter,^{†,‡} Scott E. Hensley,^{†,¶,||} Warren J. Leonard,[#] and Michael P. Cancro^{*,†}

T-bet and CD11c expression in B cells is linked with IgG_{2c} isotype switching, virus-specific immune responses, and humoral autoimmunity. However, the activation requisites and regulatory cues governing T-bet and CD11c expression in B cells remain poorly defined. In this article, we reveal a relationship among TLR engagement, IL-4, IL-21, and IFN- γ that regulates T-bet expression in B cells. We find that IL-21 or IFN- γ directly promote T-bet expression in the context of TLR engagement. Further, IL-4 antagonizes T-bet induction. Finally, IL-21, but not IFN- γ , promotes CD11c expression independent of T-bet. Using influenza virus and *Heligmosomoides polygyrus* infections, we show that these interactions function in vivo to determine whether T-bet⁺ and CD11c⁺ B cells are formed. These findings suggest that T-bet⁺ B cells seen in health and disease share the common initiating features of TLR-driven activation within this circumscribed cytokine milieu. *The Journal of Immunology*, 2016, 197: 000–000.

Although initially implicated in CD4 T cell differentiation, T-bet is a key transcriptional regulator in many immune cells. Thus, as shown in the companion report (1), B cell–intrinsic T-bet expression is required to control chronic viral infections and fosters switching to IgG_{2a} (2–4), an isotype associated with both T_H1-driven Ab responses and humoral autoimmunity (5, 6). Moreover, T-bet is required for the generation of age-associated B cells, which are transcriptionally distinct from other B cell subsets

and have also been associated with both viral clearance and humoral autoimmunity (7–9). Finally, many T-bet⁺ B cells express CD11c, a phenotype associated with viral or bacterial infections, autoimmunity, and neoplasia (8, 10–13). Despite growing appreciation for the importance of T-bet–expressing B cell subsets, the signals that yield B lineage effectors characterized by T-bet expression, as well as how these regulate appropriate versus pathogenic outcomes, remain poorly defined. Candidates include cell-intrinsic cues from adaptive and innate receptors, including the BCR and TLRs, as well as signals from T follicular helper (T_{FH}) cells. In this regard, several T_H1 cytokines, including IL-12, IL-18, and IFN- γ , can induce T-bet in activated B cells (5, 6). Nonetheless, the roles and interactions of canonical T_{FH} cell cytokines, IL-21, IL-4, and IFN- γ , in regulating T-bet expression have not been systematically interrogated (14–16).

In this article, we show that mouse and human B cells integrate signals from IL-4, IL-21, and IFN- γ to regulate T-bet expression. In the context of TLR engagement, both IL-21 and IFN- γ directly drive follicular (FO) B cells to express T-bet in vitro. However, IL-4 antagonizes IL-21–driven T-bet upregulation, but enhances IFN- γ –induced T-bet expression. Moreover, IL-21, but not IFN- γ , promotes CD11c expression. Consistent with these in vitro results, the in vivo frequencies of germinal center (GC) and memory B (B_{MEM}) cells expressing T-bet or CD11c vary based on the prevailing cytokine milieu. Finally, using viral and helminthic infections in single- and double-cytokine knockout mice, we show that the relative abundance of these cytokines determines whether GC and B_{MEM} cells generated during ongoing immune responses express T-bet and CD11c. Together, these findings

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search, National Heart, Lung, and Blood Institute, National Institutes of Health. B.B. was supported by German Research Foundation Fellowship BE5496/1-1.

The transcriptional profiling data presented in this article have been submitted to the Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE77145>) under accession number GSE77145.

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The online version of this article contains supplemental material.

Abbreviations used in this article: B6, C57BL/6; BL, BioLegend; B_{MEM}, memory B; eBio, eBioscience; FO, follicular; GC, germinal center; PR8, A/Puerto Rico/8/1934; T_{FH}, T follicular helper; VCT, Violet Cell Trace; WT, wild type.

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reveal a previously unappreciated interplay of IL-4, IL-21, and IFN- γ that, in concert with innate sensors, controls T-bet and CD11c expression in B cells.

Materials and Methods

Mice

Tbx21^{-/-}, *Stat6*^{-/-}, *Tbx21*^{fl/fl} *Cd19*^{Cre/+}, C57BL/6 (B6), and BALB/c mice were maintained and used in accordance with the University of Pennsylvania Institutional Animal Care and Use Committee guidelines. The University of Pennsylvania Institutional Animal Care and Use Committee approved all animal experiments. *Il4*^{-/-} mice were a gift from Dr. Paula Oliver. *Ifng*^{-/-} mice were a gift from Dr. Edward Behrens. *Il4*^{-/-} *Ifng*^{-/-} double-deficient mice were bred in-house. *Il21r*^{-/-} and *Il21tg* spleens and sera were shipped overnight on ice from Dr. Warren Leonard. All mice were 2–6 mo of age.

Infections

Mice were infected by oral gavage with 200 infectious larvae of *Heligmosomoides polygyrus* as previously described (17). Mice were infected by intranasal infection with 30 tissue culture infectious dose₅₀ of influenza strain A/Puerto Rico/8/1934 (PR8) (American Type Culture Collection).

In vitro cultures

Mouse CD23⁺ splenic B cells were enriched by magnetic positive selection (Miltenyi Biotec), labeled with either Violet Cell Trace (VCT; Invitrogen) or CFSE (eBioscience [eBio]), and cultured as previously described (18). Human PBMCs were isolated from blood samples obtained from healthy donors that expressed written informed consent and after ethical approval by the University of Pennsylvania Institutional Review Board. All investigations were conducted according to the principles expressed in the Declaration of Helsinki. Human B cells were enriched by CD27 microbead negative selection followed by CD19 microbead positive selection (Miltenyi Biotec), labeled with CFSE, and cultured with indicated stimuli for 5 d. Mouse or human IL-21, IL-4, and IFN- γ were used at 25, 10, and 10 ng/ml, respectively (Shenandoah). ODN2006 was used at 1 μ M (Invivogen).

Flow cytometry

FACS reagents were purchased from BioLegend (BL), BD Biosciences, or eBio: T-bet (4B10; BL), CD11c (N418; BL), IgM (R6-60.2; BD Biosciences), CD38 (90; eBio), CD138 (281-2; BL), IgD (11-26c.2a; BL), CD4 (RM4-5; BL), B220 (RA3-6B2; BL), CD62L (MEL-14; eBio), TCR- β (H57-597; BL), CD19 (6D5; BL), CXCR5 (L138D7; BL), PD-1 (RMP1-30; BL), CD8 (53-6.7; eBio), CD4 (H129.19; BL), F4/80 (BM8; eBio), Ly-6G/GR1 (RB6-8C5; eBio), CD43 (S7; BD Biosciences), CD21/CD35 (CR2/CR1; BL), CD23 (B3B4; eBio), CD93 (AA4.1; BL); peanut agglutinin-FITC (Sigma); Zombie Aqua (BL). FACS analyses were performed as described previously (18).

Serum Ab titers

ELISAs were performed as previously described (18) using anti-mouse IgG_{2a}, IgG_{2b}, IgG_{2c}, or IgG₁ HRP Abs (Southern Biotech).

Quantitative PCR analysis and transcriptional profiling

Quantitative PCR experiments were performed as previously published (18) using the following probes: *Il4* (Mm00445260_m1), *Ifng* (Mm00801778_m1), *Il21* (Mm00517640_m1), *Tbx21* (Mm00450960_m1), *Aicda* (Mm00507774_m1). Transcriptional profiling data were generated as previously described (19) and have been deposited in the Gene Expression Omnibus database for public access (accession no. GSE77145; <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE77145>).

Statistics

Student *t* test was used to generate all *p* values: **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001. Data are represented as box and whisker plots with mean depicted with plus sign (+).

Results and Discussion

IL-21, IL-4, and IFN- γ differentially regulate T-bet and CD11c expression

In preliminary in vitro studies, we established that IL-21 drives T-bet expression in mouse FO B cells responding to TLR9, but not BCR or CD40 signals (Fig. 1A). To explore these interactions further, we cultured FO B cells with IL-4, IL-21, or

IFN- γ in the presence of TLR7 or TLR9 agonists. Both *Tbx21* transcripts and T-bet protein increased markedly in FO B cells cultured with IL-21 or IFN- γ , but IL-4 influenced these outcomes differently. IL-4 blocked IL-21-driven T-bet upregulation, but enhanced IFN- γ -mediated T-bet upregulation (Fig. 1B, Supplemental Fig. 1A).

To determine whether IL-21 and IL-4 directly regulate T-bet in B cells, either *Il21r*^{-/-} or *Stat6*^{-/-} B cells were cocultured with wild type (WT) B cells and stimulated as described earlier. Because IL-21R is required for IL-21 signaling and STAT6 is the key signal transducer of IL-4 (20, 21), we reasoned that coculturing these mutants with WT cells would reveal any secondary *trans* effects. To track both cell origin and division, we labeled WT or knockout cells with VCT or CFSE, respectively (Supplemental Fig. 1B). Whereas IL-21 induced T-bet expression in WT B cells, the cocultured *Il21r*^{-/-} B cells remained T-bet⁻ (Fig. 1C, top row). Analogously, although IL-21-driven T-bet upregulation in WT B cells was reversed by IL-4, cocultured *Stat6*^{-/-} cells were refractory to this negative effect (Fig. 1C, bottom row). Similar results were obtained using the TLR7 agonist, CL097 (data not shown). Importantly, in all cases, IFN- γ treatment induced T-bet irrespective of *Il21r* or *Stat6* deficiency (Fig. 1C). To assess whether similar relationships exist in human

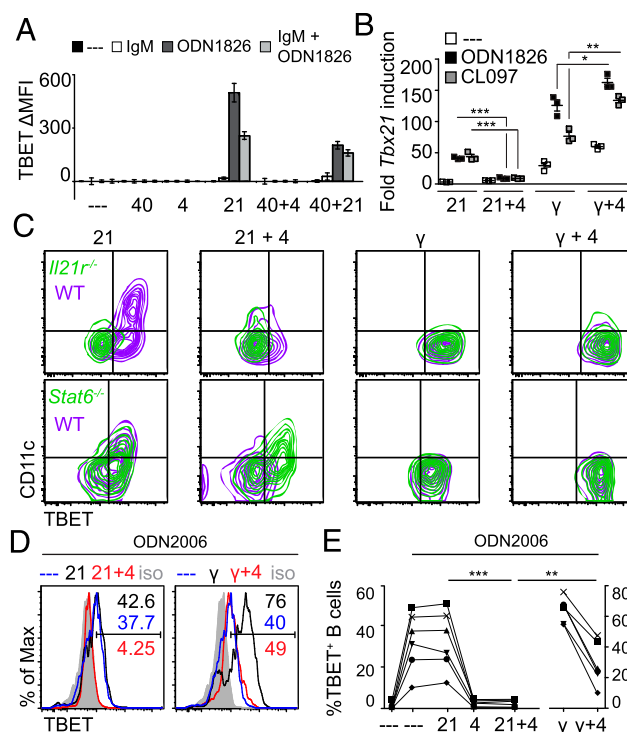


FIGURE 1. IL-4 and IL-21 act in a cell-intrinsic manner to regulate T-bet expression in vitro. Magnetically enriched CD23⁺ splenic B cells were cultured in vitro with various combinations of anti-Ig- μ (IgM), anti-CD40 (40), IL-4 (4), IL-21 (21), and IFN- γ (γ). Mouse data are representative of three independent experiments. (A) WT or *Cd19*^{Cre/+} *Tbx21*^{fl/fl} B cells treated for 48 h and assessed for T-bet mean fluorescent intensity (Δ MFI = WT – mutant). (B) *Tbx21* mRNA levels in WT cells treated for 20 h. (C) WT, *Il21r*^{-/-}, or *Stat6*^{-/-} B cells were labeled with either CFSE (green plots) or VCT (purple plots), treated with ODN1826 and indicated cytokines for 48 h, and then stained for CD11c and T-bet. (D) Magnetically enriched CD23⁺CD19⁺ human B cells were labeled with CFSE, treated for 108 h, and probed for T-bet on live CFSE⁺ cells. (E) Frequency of T-bet⁺ B cells from each treatment across six healthy adult donors. **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

B cells, we cultured CD27⁺CD19⁺ PBMCs as described earlier. TLR9 stimulation alone upregulated T-bet in these cultures. It is not clear whether intrinsic effects of TLR signaling or *trans* effects induced by these signals underlie this observation. Nonetheless, IFN- γ significantly increased T-bet expression, and IL-4 completely blocked T-bet in all cultures except those with IFN- γ (Fig. 1D, 1E). In toto, these results show that in the context of TLR signaling, IL-4, IL-21, and IFN- γ interact to regulate T-bet expression in both mouse and human B cells.

The converse effect of IL-4 on IFN- γ versus IL-21-induced T-bet expression suggests that unique, T-bet-associated programs are facilitated by each cytokine. We interrogated this possibility in several ways. First, because previous studies have linked T-bet with CD11c expression (8), we asked whether IFN- γ or IL-21 influence CD11c differently. The results show that IL-21 drives CD11c expression, but IFN- γ does not (Fig. 1C). Further, as with T-bet, IL-4 blocks IL-21-induced CD11c expression. Finally, IFN- γ drives T-bet expression and is not appreciably influenced by either IL-4 or IL-21 (Supplemental Fig. 1C). These findings indicate that IL-21 and IFN- γ drive T-bet and CD11c expression through distinct mediators, and that T-bet expression per se is insufficient for CD11c induction. To further interrogate differential T-bet expression driven by IL-21 versus IFN- γ , as well as to distinguish T-bet-dependent and -independent effects of each cytokine, we performed genome-wide transcriptional profiling on WT or *Tbx21*^{-/-} B cells stimulated with either IFN- γ or IL-21. Principal components analysis shows that 82.7% of variance in these data was explained by the cytokine used, whereas *Tbx21* genotype accounted for 6.3% of the variance (Supplemental Fig. 1D). Further, each cytokine induces a unique transcriptional profile, including some T-bet-dependent shifts in gene expression (Supplemental Fig. 1E, Supplemental Table I). Thus, IFN- γ and IL-21 drive similar but distinct T-bet-associated phenotypes in B cells.

Together, these results show that in the context of TLR engagement, the aggregate of IFN- γ , IL-21, and IL-4 signals determines whether B cells express T-bet. TLR engagement, but not BCR cross-linking (Fig. 1A), appears necessary to position B cells for T-bet expression upon subsequent IFN- γ or IL-21 signaling. We obtained similar results with the TLR2/4 ligand LPS (not shown), suggesting pathways common to most TLRs, and perhaps other innate receptors, provide these key initial signals. We speculate that these signals alter gene loci accessibility for subsequent cytokine cues. Indeed, prior reports that CD11c⁺ or T-bet⁺ B cells emerge in response to a variety of viral and bacterial infections are consistent with this idea (7, 10). Moreover, the differential effects of IL-4 on IL-21 versus IFN- γ suggest a complex interplay of STAT-dependent transcriptional regulation. The clear dose-response relationship of IL-4-mediated effects is consistent with the idea that competitive relationships are involved (Supplemental Fig. 1F). Although IL-4 and IL-21 both require common γ -chain receptor to initiate STAT signal transduction (22), our *Stat6*^{-/-} coculture data (Fig. 1C) indicate that competition for membrane proximal receptor components is unlikely to explain these findings. If this were the case, then *Stat6*^{-/-} cells would also be subject to the repressive effects of IL-4. Instead, downstream events are

more likely candidates, including differential occupation of transcriptional regulatory sites and altered stoichiometric relationships among the JAK-STAT proteins involved.

Relative abundance of IL-21, IL-4, and IFN- γ regulates T-bet expression in vivo

Our in vitro findings suggest that IFN- γ , IL-4, and IL-21 interact to modulate T-bet and CD11c expression in B cells. As an initial assessment of whether this relationship exists in vivo, we surveyed GC B and B_{MEM} cells for T-bet expression in B6 versus BALB/c mice (Supplemental Fig. 1G), because these strains display inherent T_H1 versus T_H2 skewing, respectively (23). We reasoned that if T-bet expression is promoted by milieu rich in IFN- γ , but repressed in those with plentiful IL-4 and little IFN- γ , then the frequencies of T-bet⁺ B cells in these two strains should differ. In agreement with this prediction, whereas most GC B cells in B6 mice are T-bet⁺, BALB/c have a lower frequency of T-bet⁺ GC B cells (Fig. 2A). Importantly, CD11c protein expression was restricted to B6 B_{MEM} cells (Fig. 2B) and not GC B cells (Supplemental Fig. 1H). These findings are consistent with the notion that IFN- γ and IL-4 levels regulate T-bet expression in GC B cells. To probe the impact of IL-21 on this overall relationship, we next asked whether extraphysiological levels of IL-21 would foster accumulation of T-bet⁺CD11c⁺ B cells. Profound increases in both T-bet and CD11c expression were seen in all splenic B cells in *Il21tg* mice (Fig. 2C), which is consistent with our in vitro results suggesting that IL-21 drives both T-bet and CD11c expression. Although the partially activated state of B cells in these mice confounds conventional phenotyping strategies, nearly all mature B cells in the *Il21tg* bear a CD23⁺CD21⁺ phenotype (Supplemental Fig. 1I) identical to the T-bet-dependent age-associated B cell subset (18, 24). Finally, consistent with the role of T-bet in fostering class-switch recombination to IgG_{2a/c}, we observed a marked increase of

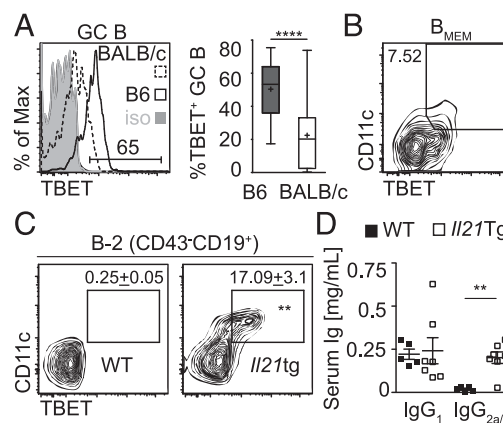


FIGURE 2. T-bet⁺CD11c⁺ cells delineate a B_{MEM} cell subset and accumulate in *Il21tg* mice. (A and B) GC B and B_{MEM} cells were analyzed for T-bet and CD11c expression by FACS. GC B and B_{MEM} cell gating strategies are in Supplemental Fig. 1G. All panels are representative of three independent experiments with ≥ 3 mice per strain. (A) T-bet staining on GC B cells from B6 ($n = 14$) or BALB/c ($n = 23$) mice with frequency enumeration. (B) T-bet and CD11c staining on B_{MEM} cells from B6 mice. (C) T-bet and CD11c staining on splenic B-2 cells from WT and *Il21tg* mice. (D) Serum IgG₁ or IgG_{2a/c} (IgG_{2a} + IgG_{2c}) levels in WT and *Il21tg* mice were determined by ELISA. Values are means \pm SEM from five WT and seven *Il21tg* mice. ** $p < 0.01$, **** $p < 0.0001$.

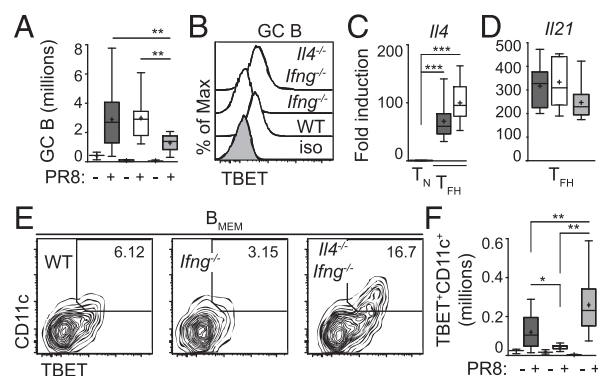


FIGURE 3. Influenza virus infection drives T-bet⁺CD11c⁺ B_{MEM} cell formation in the absence of both IFN- γ and IL-4. Splenocytes were harvested from noninfected (–) or day 10 after intranasal 30 tissue culture infectious dose₅₀ PR8 infection (+) WT ($n = 21$, black bars), *Ifng*^{–/–} ($n = 10$, white bars), or *Il4*^{–/–}*Ifng*^{–/–} ($n = 13$, gray bars) mice across 3–7 experiments with ≥ 3 mice per group. GC B, B_{MEM}, and T_{FH} cell gating strategies are in Supplemental Fig. 1G and 1J. (A) Enumeration of GC B cells. (B) Tbet staining on GC B cells. (C) *Il4* and (D) *Il21* mRNA levels from sorted naive CD62L⁺ CD4 T (T_N, $n = 9$) or T_{FH} cells. (E) Proportions and (F) numbers of T-bet⁺CD11c⁺ B_{MEM} cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

IgG_{2a/c}, but not IgG₁, serum Ab titers in *Il21*tg compared with WT mice (Fig. 2D).

Together, our in vitro and in vivo observations prompt a model in which the relative availability of IL-4, IL-21, and IFN- γ governs the likelihood of establishing B_{MEM} cells expressing Tbet and CD11c. Further, they suggest that abundant IFN- γ will drive a Tbet⁺CD11c[–] phenotype regardless of IL-4 or IL-21 levels, but that in the absence of IFN- γ , the Tbet⁺CD11c⁺ phenotype is reciprocally regulated by IL-21 versus IL-4. We therefore evaluated these predictions by tracking the immune responses to either influenza virus or *H. polygyrus* in mice where cytokine availability could be experimentally manipulated.

Influenza virus infection generates Tbet⁺CD11c⁺ B_{MEM} in the absence of both IL-4 and IFN- γ

Influenza virus infection yields a well-characterized T-dependent and T_H1-skewed response, in which responding T_{FH} cells produce copious IFN- γ , as well as IL-21 and IL-4 (14). Thus, we reasoned that IFN- γ would induce Tbet expression in GC B and B_{MEM} cells, but in the absence of IFN- γ , IL-4 would prevent Tbet expression. Accordingly, WT or *Ifng*^{–/–} mice were infected with the influenza virus strain PR8. As expected, WT animals mounted a robust GC B cell response to PR8 (Fig. 3A), and these GC B cells expressed Tbet (Fig. 3B; sort strategy and *Tbx21* expression, Supplemental Fig. 1J, 1K). In contrast, GC B cells in *Ifng*^{–/–} mice failed to express Tbet even though the magnitude of the GC B cell response was similar to WT. Assuming that T_{FH} cells are the major source of cytokine, we confirmed that both WT and *Ifng*^{–/–} mice made substantial numbers of T_{FH} cells (Supplemental Fig. 1J, 1L), and their capacity to make IL-4 and IL-21 was unperturbed (Fig. 3C, 3D). These results are consistent with the idea that, in the absence of IFN- γ , IL-4 blocks Tbet expression in response to IL-21. To directly test this, we infected *Il4*^{–/–}*Ifng*^{–/–} double-deficient mice with PR8. Although *Il4*^{–/–}*Ifng*^{–/–} mice mounted a blunted GC B cell response (Fig. 3A), these cells nonetheless express Tbet (Fig. 3B, Supplemental Fig. 1K).

Although the splenic plasma cell numbers were reduced in *Ifng*^{–/–} mice, B_{MEM} cell numbers remained intact across genotypes (Supplemental Fig. 1M, 1N). However, the composition of the B_{MEM} cell pool differed according to genotype (Fig. 3E, 3F). Whereas WT mice generated some Tbet⁺CD11c⁺ B_{MEM} cells, *Ifng*^{–/–} mice produced few, if any, above noninfected control animals, likely reflecting the dominance of IL-4 in the absence of IFN- γ . Consistent with this interpretation, *Il4*^{–/–}*Ifng*^{–/–} mice generated the most Tbet⁺CD11c⁺ B_{MEM} cells. Lastly, CD11c expression was restricted to B_{MEM} cells and not GC B cells (Supplemental Fig. 1O). Overall, these findings confirm and extend our in vitro findings, because the same interplay of cytokines directs Tbet expression among B effectors in vivo. Further, our observations suggest that Tbet⁺CD11c⁺ B_{MEM} cells will be fostered in immune responses where IL-4 is limited.

*Il4 deficiency yields Tbet⁺CD11c⁺ B_{MEM} independent of IFN- γ in *H. polygyrus* infection*

Results with influenza virus infection are consistent with the notion that IFN- γ drives Tbet expression irrespective of concomitant IL-4 or IL-21, and that eliminating IFN- γ creates a situation where the relative levels of IL-4 and IL-21 govern the Tbet⁺CD11c⁺ phenotype. However, this subtractive approach does not necessarily show that, in responses where IFN- γ is normally absent, the sole determinant of Tbet expression is IL-4 availability. Accordingly, we asked whether IL-4 deficiency is sufficient to permit Tbet expression in GC B and B_{MEM} cells during a T_H2 response, using *H. polygyrus*. This intestinal helminth induces IL-4 and IL-21 production by T_{FH} cells, which drives a robust IgG₁ response (15). Thus, we hypothesized that, in the absence of IL-4, IL-21 would be sufficient to induce Tbet expression in GC B and B_{MEM} cells. To test this idea, we infected WT or *Il4*^{–/–} mice with *H. polygyrus* and probed GC B cells for Tbet. As expected, WT mice mounted a GC B cell response that lacked Tbet expression, which correlated with increased serum IgG₁ titers. Conversely, although blunted in magnitude,

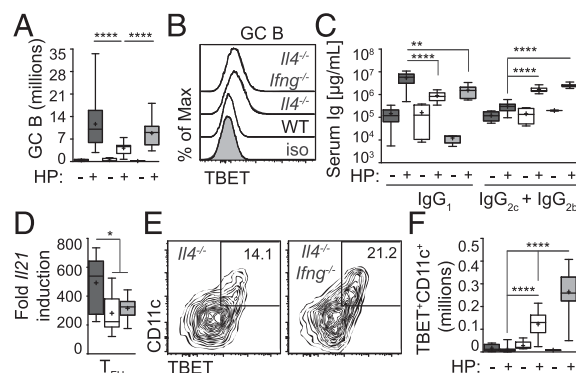


FIGURE 4. Activated B cells express Tbet independent of IFN- γ in IL-4 limiting conditions. Splenocytes and sera were harvested from noninfected (–) or day 14 after oral gavage (+) of 200 *H. polygyrus* in WT ($n = 20$, black bars), *Il4*^{–/–} ($n = 24$, white bars), or *Il4*^{–/–}*Ifng*^{–/–} ($n = 11$, gray bars) mice across 3–6 experiments with ≥ 3 mice per group. GC B, B_{MEM}, and T_{FH} cell gating strategies are in Supplemental Fig. 1G and 1J. (A) Enumeration of GC B cells. (B) Tbet staining on GC B cells. (C) Serum concentrations of IgG₁ and IgG_{2c} + IgG_{2b}. (D) *Il21* mRNA levels from sorted T_{FH} cells. (E) Proportions and (F) numbers of Tbet⁺CD11c⁺ B_{MEM} cells. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

$IL4^{-/-}$ mice initiated a T-bet⁺ GC B cell response with decreased serum IgG₁ titers compared with WT (Fig. 4A–C, Supplemental Fig. 1J, 1P). To eliminate the possibility that excess IFN- γ in $IL4^{-/-}$ mice explains these phenotypes, we infected $IL4^{-/-} Ifng^{-/-}$ mice with *H. polygyrus*. The GC B cell response in $IL4^{-/-} Ifng^{-/-}$ mice was similar to WT levels (Fig. 4A) but maintained T-bet expression independently of IFN- γ (Fig. 4B, Supplemental Fig. 1J, 1P). Isotype representation varied with T-bet expression: whereas WT mice produced >95% IgG₁, more than half of the serum Abs in $IL4^{-/-} Ifng^{-/-}$ and $IL4^{-/-}$ mice were IgG_{2b} and IgG_{2c} (Fig. 4C). Further, whereas $IL4^{-/-} Ifng^{-/-}$ mice mounted a higher T_{FH} cell response (Supplemental Fig. 1Q), both $IL4^{-/-}$ and $IL4^{-/-} Ifng^{-/-}$ mice produced less IL-21 (Fig. 4D). Regardless, the magnitude of the plasma cell and B_{MEM} cell response remained intact across genotypes (Supplemental Fig. 1R, 1S). However, we again observed alterations in the B_{MEM} pool according to cytokine availability. Whereas *H. polygyrus*-infected WT mice did not generate T-bet⁺CD11c⁺ B_{MEM} cells, both $IL4^{-/-}$ and $IL4^{-/-} Ifng^{-/-}$ mice did, again suggesting IL-21 drives a unique T-bet⁺ phenotype (Fig. 4E, 4F). Whereas prior reports showed CD11c mRNA in GC B cells defined by CD95 and peanut agglutinin (25), we observed CD11c protein expression only in B_{MEM} cells (Supplemental Fig. 1T). This seeming disparity may indicate that CD11c transcripts in GC B cells go untranslated, as well as the further resolution of GC and B_{MEM} by CD38 in our gating strategy. Overall, the *H. polygyrus* infection data support our model, inasmuch as in the absence of IFN- γ we observe both T-bet and CD11c expression that is modulated by IL-4. Further, the consistent relationships observed in both types of infection argue that this is a feature common to most humoral immune responses.

In toto, our findings reveal a novel cytokine network that governs T-bet expression in the context of TLR stimulation. In the absence of IFN- γ , IL-4 and IL-21 reciprocally regulate T-bet and CD11c expression both in vitro and in vivo. Because immune responses are rarely monolithic with regard to these three cytokines (14, 26), distinct or multifunctional T_{FH} cells likely generate a diverse set of B effectors. Consequently, altering the cytokine milieu affects the isotypes generated (Fig. 4C) and the composition of the B_{MEM} pools (Figs. 3F, 4F) while maintaining the magnitude of the response.

It is tempting to speculate that the T-bet⁺CD11c⁺ B cells reported in autoimmunity, viral infections, and aging share a common underlying origin involving TLR engagement coupled with either copious IFN- γ or abundant IL-21 with little IL-4. Indeed, both TLR7 and IL-21 deficiencies ameliorate disease in humoral autoimmunity models (27, 28), and poor IL-4 production has been observed in T_{FH} cells from aged mice (29). Thus, understanding this interplay among IL-4, IL-21, and IFN- γ might better define the etiology of humoral autoimmune syndromes where such cells are implicated (8, 13, 30). Lastly, although it is clear that IFN- γ and IL-21 differentially induce CD11c expression (Fig. 1C), the functional consequences of expressing this integrin remain elusive. Importantly, the restriction of CD11c expression to B_{MEM} cells is consistent with prior B_{MEM} subsetting studies in human tonsils and may thus define a tissue-homing population (31). Accordingly, further studies are needed to assess the role of these different T-bet⁺ B_{MEM} cells in both health and disease.

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Disclosures

The authors have no financial conflicts of interest.

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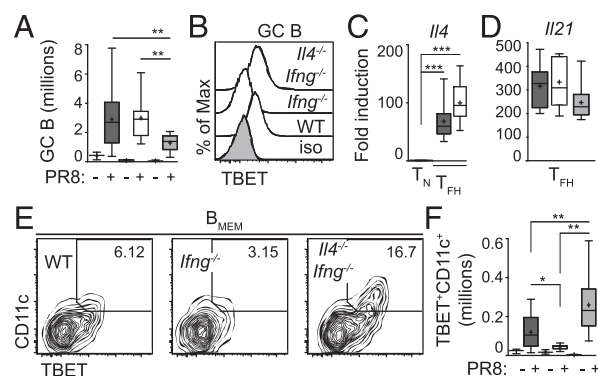


FIGURE 3. Influenza virus infection drives T-bet⁺CD11c⁺ B_{MEM} cell formation in the absence of both IFN- γ and IL-4. Splenocytes were harvested from noninfected (–) or day 10 after intranasal 30 tissue culture infectious dose₅₀ PR8 infection (+) WT ($n = 21$, black bars), *Ifng*^{–/–} ($n = 10$, white bars), or *Il4*^{–/–}*Ifng*^{–/–} ($n = 13$, gray bars) mice across 3–7 experiments with ≥ 3 mice per group. GC B, B_{MEM}, and T_{FH} cell gating strategies are in Supplemental Fig. 1G and 1J. (A) Enumeration of GC B cells. (B) Tbet staining on GC B cells. (C) *Il4* and (D) *Il21* mRNA levels from sorted naive CD62L⁺ CD4 T (T_N, $n = 9$) or T_{FH} cells. (E) Proportions and (F) numbers of T-bet⁺CD11c⁺ B_{MEM} cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

IgG_{2a/c}, but not IgG₁, serum Ab titers in *Il21*tg compared with WT mice (Fig. 2D).

Together, our in vitro and in vivo observations prompt a model in which the relative availability of IL-4, IL-21, and IFN- γ governs the likelihood of establishing B_{MEM} cells expressing Tbet and CD11c. Further, they suggest that abundant IFN- γ will drive a Tbet⁺CD11c[–] phenotype regardless of IL-4 or IL-21 levels, but that in the absence of IFN- γ , the Tbet⁺CD11c⁺ phenotype is reciprocally regulated by IL-21 versus IL-4. We therefore evaluated these predictions by tracking the immune responses to either influenza virus or *H. polygyrus* in mice where cytokine availability could be experimentally manipulated.

Influenza virus infection generates Tbet⁺CD11c⁺ B_{MEM} in the absence of both IL-4 and IFN- γ

Influenza virus infection yields a well-characterized T-dependent and T_H1-skewed response, in which responding T_{FH} cells produce copious IFN- γ , as well as IL-21 and IL-4 (14). Thus, we reasoned that IFN- γ would induce Tbet expression in GC B and B_{MEM} cells, but in the absence of IFN- γ , IL-4 would prevent Tbet expression. Accordingly, WT or *Ifng*^{–/–} mice were infected with the influenza virus strain PR8. As expected, WT animals mounted a robust GC B cell response to PR8 (Fig. 3A), and these GC B cells expressed Tbet (Fig. 3B; sort strategy and *Tbx21* expression, Supplemental Fig. 1J, 1K). In contrast, GC B cells in *Ifng*^{–/–} mice failed to express Tbet even though the magnitude of the GC B cell response was similar to WT. Assuming that T_{FH} cells are the major source of cytokine, we confirmed that both WT and *Ifng*^{–/–} mice made substantial numbers of T_{FH} cells (Supplemental Fig. 1J, 1L), and their capacity to make IL-4 and IL-21 was unperturbed (Fig. 3C, 3D). These results are consistent with the idea that, in the absence of IFN- γ , IL-4 blocks Tbet expression in response to IL-21. To directly test this, we infected *Il4*^{–/–}*Ifng*^{–/–} double-deficient mice with PR8. Although *Il4*^{–/–}*Ifng*^{–/–} mice mounted a blunted GC B cell response (Fig. 3A), these cells nonetheless express Tbet (Fig. 3B, Supplemental Fig. 1K).

Although the splenic plasma cell numbers were reduced in *Ifng*^{–/–} mice, B_{MEM} cell numbers remained intact across genotypes (Supplemental Fig. 1M, 1N). However, the composition of the B_{MEM} cell pool differed according to genotype (Fig. 3E, 3F). Whereas WT mice generated some Tbet⁺CD11c⁺ B_{MEM} cells, *Ifng*^{–/–} mice produced few, if any, above noninfected control animals, likely reflecting the dominance of IL-4 in the absence of IFN- γ . Consistent with this interpretation, *Il4*^{–/–}*Ifng*^{–/–} mice generated the most Tbet⁺CD11c⁺ B_{MEM} cells. Lastly, CD11c expression was restricted to B_{MEM} cells and not GC B cells (Supplemental Fig. 1O). Overall, these findings confirm and extend our in vitro findings, because the same interplay of cytokines directs Tbet expression among B effectors in vivo. Further, our observations suggest that Tbet⁺CD11c⁺ B_{MEM} cells will be fostered in immune responses where IL-4 is limited.

*Il4 deficiency yields Tbet⁺CD11c⁺ B_{MEM} independent of IFN- γ in *H. polygyrus* infection*

Results with influenza virus infection are consistent with the notion that IFN- γ drives Tbet expression irrespective of concomitant IL-4 or IL-21, and that eliminating IFN- γ creates a situation where the relative levels of IL-4 and IL-21 govern the Tbet⁺CD11c⁺ phenotype. However, this subtractive approach does not necessarily show that, in responses where IFN- γ is normally absent, the sole determinant of Tbet expression is IL-4 availability. Accordingly, we asked whether IL-4 deficiency is sufficient to permit Tbet expression in GC B and B_{MEM} cells during a T_H2 response, using *H. polygyrus*. This intestinal helminth induces IL-4 and IL-21 production by T_{FH} cells, which drives a robust IgG₁ response (15). Thus, we hypothesized that, in the absence of IL-4, IL-21 would be sufficient to induce Tbet expression in GC B and B_{MEM} cells. To test this idea, we infected WT or *Il4*^{–/–} mice with *H. polygyrus* and probed GC B cells for Tbet. As expected, WT mice mounted a GC B cell response that lacked Tbet expression, which correlated with increased serum IgG₁ titers. Conversely, although blunted in magnitude,

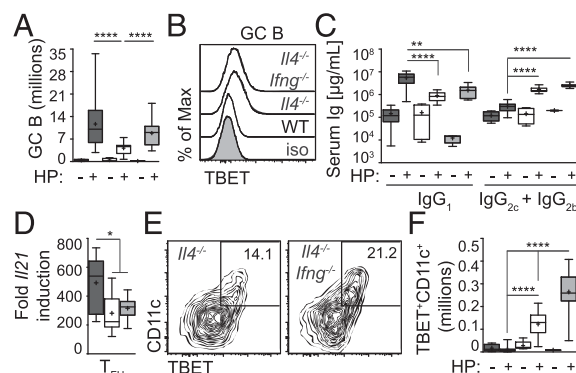


FIGURE 4. Activated B cells express Tbet independent of IFN- γ in IL-4 limiting conditions. Splenocytes and sera were harvested from noninfected (–) or day 14 after oral gavage (+) of 200 *H. polygyrus* in WT ($n = 20$, black bars), *Il4*^{–/–} ($n = 24$, white bars), or *Il4*^{–/–}*Ifng*^{–/–} ($n = 11$, gray bars) mice across 3–6 experiments with ≥ 3 mice per group. GC B, B_{MEM}, and T_{FH} cell gating strategies are in Supplemental Fig. 1G and 1J. (A) Enumeration of GC B cells. (B) Tbet staining on GC B cells. (C) Serum concentrations of IgG₁ and IgG_{2c} + IgG_{2b}. (D) *Il21* mRNA levels from sorted T_{FH} cells. (E) Proportions and (F) numbers of Tbet⁺CD11c⁺ B_{MEM} cells. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

$IL4^{-/-}$ mice initiated a T-bet⁺ GC B cell response with decreased serum IgG₁ titers compared with WT (Fig. 4A–C, Supplemental Fig. 1J, 1P). To eliminate the possibility that excess IFN- γ in $IL4^{-/-}$ mice explains these phenotypes, we infected $IL4^{-/-} Ifng^{-/-}$ mice with *H. polygyrus*. The GC B cell response in $IL4^{-/-} Ifng^{-/-}$ mice was similar to WT levels (Fig. 4A) but maintained T-bet expression independently of IFN- γ (Fig. 4B, Supplemental Fig. 1J, 1P). Isotype representation varied with T-bet expression: whereas WT mice produced >95% IgG₁, more than half of the serum Abs in $IL4^{-/-} Ifng^{-/-}$ and $IL4^{-/-}$ mice were IgG_{2b} and IgG_{2c} (Fig. 4C). Further, whereas $IL4^{-/-} Ifng^{-/-}$ mice mounted a higher T_{FH} cell response (Supplemental Fig. 1Q), both $IL4^{-/-}$ and $IL4^{-/-} Ifng^{-/-}$ mice produced less IL-21 (Fig. 4D). Regardless, the magnitude of the plasma cell and B_{MEM} cell response remained intact across genotypes (Supplemental Fig. 1R, 1S). However, we again observed alterations in the B_{MEM} pool according to cytokine availability. Whereas *H. polygyrus*-infected WT mice did not generate T-bet⁺CD11c⁺ B_{MEM} cells, both $IL4^{-/-}$ and $IL4^{-/-} Ifng^{-/-}$ mice did, again suggesting IL-21 drives a unique T-bet⁺ phenotype (Fig. 4E, 4F). Whereas prior reports showed CD11c mRNA in GC B cells defined by CD95 and peanut agglutinin (25), we observed CD11c protein expression only in B_{MEM} cells (Supplemental Fig. 1T). This seeming disparity may indicate that CD11c transcripts in GC B cells go untranslated, as well as the further resolution of GC and B_{MEM} by CD38 in our gating strategy. Overall, the *H. polygyrus* infection data support our model, inasmuch as in the absence of IFN- γ we observe both T-bet and CD11c expression that is modulated by IL-4. Further, the consistent relationships observed in both types of infection argue that this is a feature common to most humoral immune responses.

In toto, our findings reveal a novel cytokine network that governs T-bet expression in the context of TLR stimulation. In the absence of IFN- γ , IL-4 and IL-21 reciprocally regulate T-bet and CD11c expression both in vitro and in vivo. Because immune responses are rarely monolithic with regard to these three cytokines (14, 26), distinct or multifunctional T_{FH} cells likely generate a diverse set of B effectors. Consequently, altering the cytokine milieu affects the isotypes generated (Fig. 4C) and the composition of the B_{MEM} pools (Figs. 3F, 4F) while maintaining the magnitude of the response.

It is tempting to speculate that the T-bet⁺CD11c⁺ B cells reported in autoimmunity, viral infections, and aging share a common underlying origin involving TLR engagement coupled with either copious IFN- γ or abundant IL-21 with little IL-4. Indeed, both TLR7 and IL-21 deficiencies ameliorate disease in humoral autoimmunity models (27, 28), and poor IL-4 production has been observed in T_{FH} cells from aged mice (29). Thus, understanding this interplay among IL-4, IL-21, and IFN- γ might better define the etiology of humoral autoimmune syndromes where such cells are implicated (8, 13, 30). Lastly, although it is clear that IFN- γ and IL-21 differentially induce CD11c expression (Fig. 1C), the functional consequences of expressing this integrin remain elusive. Importantly, the restriction of CD11c expression to B_{MEM} cells is consistent with prior B_{MEM} subsetting studies in human tonsils and may thus define a tissue-homing population (31). Accordingly, further studies are needed to assess the role of these different T-bet⁺ B_{MEM} cells in both health and disease.

Acknowledgments

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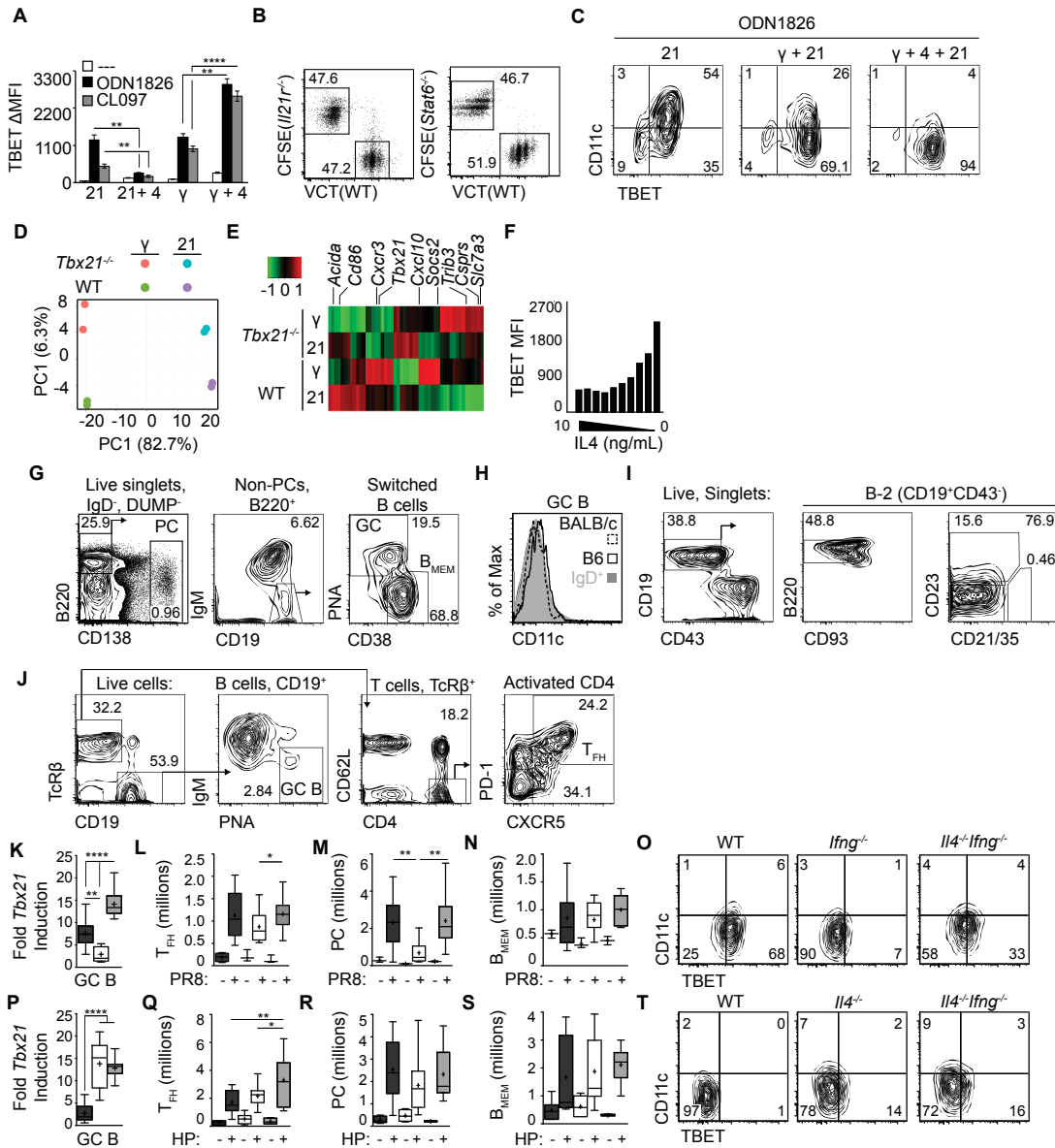
Disclosures

The authors have no financial conflicts of interest.

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Supplemental Figure 1: Treatment abbreviation: IL4 (4), IL21 (21), IFN γ (γ). **(A)** TBET protein (Δ MFI=WT-isotype) in WT FO B cells treated for 48h. **(B)** WT, *Il21*^{-/-}, or *Stat6*^{-/-} FO B cells labeled with either CFSE or Violet Cell Trace (VCT), treated with ODN1826. **(C)** TBET and CD11c protein on WT FO B cells treated for 48h. **(D)** Principal component analysis and **(E)** heat map of top 50 differentially regulated genes from WT or *Tbx21*^{-/-} FO B cells cultured with ODN1826 and IFN γ or IL21 for 20h; 2 biological replicates per condition from one experiment. **(F)** IL4 titration on ODN1826 and IL21 stimulated B cells probed for TBET. **(G)** Gating strategy for splenic PCs, GC B, and B_{MEM} cells. DUMP is defined as CD4, CD8, F4/80, and Gr1. **(H)** CD11c staining on GC B cells from B6 or BALB/c mice. **(I)** FACS analysis for pre-immune B cell subsets in *Il21*Tg spleens. **(J)** Representative sorting strategy for T_{FH} and GC B cells during infection for all RNA and T_{FH} cell numbers. **(K-O)** Data shown are from PR8-infected mice (WT black bars, *Ilfng*^{-/-} white bars, *Il4*^{-/-}*Ilfng*^{-/-} grey bars) as described in Fig. 3. **(P-T)** Data shown are from HP-infected mice (WT black bars, *Il4*^{-/-} white bars, *Il4*^{-/-}*Ilfng*^{-/-} grey bars) as described in Fig. 4. **(K&P)** *Tbx21* gene expression from sorted GC B cells is shown. Splenic cell counts for **(L&Q)** T_{FH}, **(M&R)** PCs, **(N&S)** B_{MEM} cells are shown. **(O&T)** FACS stains for TBET and CD11c on GC B cells are shown.

Supplemental Table 1: Top 50 genes differentially expressed according to stimulation and genotype that generated the heatmap in **Figure 1E**.

diffSymbols	diffEntrez	Tbx21 KO		WT	
		IFNG	IL21	IFNG	IL21
Hbb-bt	101488143	8.71787854	9.96251869	9.22367706	10.7184761
Pld4	104759	9.63100389	11.8759995	10.2329291	12.2633218
Csprs	114564	10.1736638	9.78810189	9.80904596	9.12704895
Adssl1	11565	8.38037551	9.56102674	8.75866032	10.1892954
Aicda	11628	8.15876623	9.56620638	8.16116397	10.6193638
Alas2	11656	8.07791416	8.17076737	8.70427272	8.69881681
Slc7a3	11989	12.3331489	10.8922169	11.9363609	9.58431359
Camk2b	12323	8.33696042	8.46884015	9.34572705	8.76825437
Cd86	12524	10.2058114	11.4837841	11.291188	11.9359246
Cebpb	12608	11.0258621	10.2639223	10.3954173	9.79851261
Cxcr3	12766	8.02271357	7.96206013	8.89044742	8.59224553
Dapk2	13143	9.0456691	8.29162501	10.358997	8.4225753
Ddx6	13209	11.2174672	11.0245372	10.8475324	10.4293705
Dmwd	13401	8.56273345	8.78857332	9.52515528	9.25084509
Igf2bp3	140488	8.80263258	9.18672253	9.51911174	9.90179174
Gcnt1	14537	10.2827501	10.4188283	9.69190833	10.3032536
Gfi1	14581	9.03038563	8.89669405	10.0634526	8.96113555
Slc6a9	14664	10.296786	9.31732143	9.73786153	8.70447742
Gpr65	14744	9.18001479	9.65880491	9.98113174	10.0820403
Hba-a1	15122	10.6116569	11.0756745	12.8136936	12.9819337
Hhex	15242	10.9035453	11.8746337	11.4957655	12.0253224
Cxcl10	15945	9.69740321	8.04664183	11.2630435	8.07099991
Mrc1	17533	8.44177481	8.06620528	9.20057647	8.06505932
Enpp1	18605	9.43758182	9.03088817	10.1047894	8.95693649
Lgals3bp	19039	11.3456087	10.327613	10.4720993	9.52062838
Sdc3	20970	9.22445568	9.34384651	8.48282303	8.68587941
Socs2	216233	8.39402055	8.31061723	9.21300752	8.25316026
Phf11a	219131	10.6754791	10.9923491	9.91001761	10.62874
Trib3	228775	10.9374695	10.7364234	10.3685751	9.51736281
Oas1	231655	10.0267927	9.07570675	9.41354243	8.69335125
Blvrb	233016	11.1287209	11.8152945	12.0710889	12.2488551
Rsad1	237926	8.70676248	8.35758333	9.48501461	8.97924121
Oas1g	23960	9.43862604	9.85463686	8.61867238	9.16101691
Usp18	24110	11.6695151	9.69335201	10.8173747	9.01086482
Ifnlr1	242700	10.6376498	8.60445256	10.0198364	8.45185354
Asns	27053	11.1359023	10.9173486	10.4862617	9.8579122
5031414D18Rik	271221	11.0728021	11.3432014	10.4476	11.0836712
Abi2	329165	10.767115	10.7019442	11.4634784	11.0806552
Tbx21	57765	8.50585362	8.28965615	12.0671288	10.6782958
Fkbp11	66120	9.26400071	8.93283424	9.92222723	9.10795164
Ifitm3	66141	11.6829144	10.3947567	10.5198475	9.94597162
Serpinb1a	66222	10.415432	9.24192845	9.63859227	9.25643539
Sec11c	66286	9.74339634	9.88278761	10.3682081	10.1358717
Entpd4	67464	11.9632761	11.9573035	11.2856712	11.2526164
Chac1	69065	12.0710536	11.8189737	11.3905192	10.3380145
Tmem110	69179	9.6681036	10.0680743	10.4259964	10.2240436
Endod1	71946	10.9566634	11.4212969	10.3114004	11.339
Hvcn1	74096	10.6755837	12.3965662	11.5044663	12.5150879
Ly6k	76486	8.96899275	8.66957018	9.57076498	9.26064778
Lbh	77889	12.9253357	13.7171333	12.328088	13.5347758

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Age-associated B cells: key mediators of both protective and autoreactive humoral responses

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Abstract: A subset of B cells with unique phenotypic and functional features—termed Age-associated B cells (ABCs)—has recently been identified in both mice and humans. These cells are characterized by a T-BET driven transcriptional program, robust responsiveness to TLR7 and TLR9 ligands, and a propensity for IgG_{2a/c} production. Beyond their age-related accumulation, these cells play roles in both normal and pathogenic humoral immune responses regardless of host age. Thus, B cells with the ABC phenotype and transcriptional signature appear during viral, bacterial, and parasitic infections, but also arise during humoral autoimmune disease in both mouse models and humans. These observations suggest that both autoantigens and certain classes of pathogens provide the signals required for ABC differentiation. Herein, we review the discovery and features of ABCs, and propose that they are a memory subset generated by nucleic acid-containing antigens in the context of a promoting inflammatory cytokine milieu.

Keywords: Age-associated B cell, autoimmunity, T-BET, TLR7, TLR9, CD11c

Introduction

Protective immunity relies on establishing appropriate lymphocyte effector functions during primary responses, then sustaining these qualities in effector and memory lymphocyte pools. While correctly tailored effector functions promote lifelong immunity, inappropriate effector choices can yield failed pathogen clearance, chronic inflammation, or autoimmunity. Importantly, although the specificity of adaptive immune responses is achieved via the T- and B-cell antigen receptors (TCRs and BCRs), the quality of subsequently generated effectors is guided by further cues that reflect the context of ongoing host–pathogen interactions. For example, conserved pathogen-associated molecular patterns (PAMPs) engage various pattern recognition receptors (PRRs), whose signals initiate differentiative programs that direct particular effector choices (reviewed in 1). Similarly, cytokines and costimulatory molecules induced through these interactions can further focus and modulate effector differentiation. Master transcriptional regulators that reinforce particular fates

frequently characterize these effector differentiation patterns. Thus, pre-immune T cells adopt alternative effector fates based on TCR signal strength (2, 3), costimulatory cues (4, 5), and cytokine milieu. Multiple T-cell effector and memory lineages are now well established, with a corresponding appreciation of the master transcriptional regulators fostering each fate (reviewed in 6–8).

Analogously, B cells can adopt a variety of distinct effector fates that are guided by BCR signal strength coupled with additional cues that foster key transcriptional programs. These include antibody production, antigen presentation and costimulatory interactions with CD4 T cells, and cytokine secretion. Among these, the nature, levels, and persistence of immunoglobulin heavy chain isotypes that are provoked have profound implications for response quality, inasmuch as these features largely dictate the spectrum of effector functions that will be established. Indeed, nearly all heavy chain isotypes link adaptive humoral responses with a circumscribed group of innate inflammatory cells and systems important for pathogen clearance, such as complement fixation, opsonization, degranulation, and antibody-dependent cell-mediated cytotoxicity (ADCC). Many of these effector processes rely on isotype-specific Fc receptors that are differentially distributed among innate, myeloid, and other cell types, which can either enhance or limit the activation and behavior of these cells (reviewed in 9). While marshaling these potent effector systems is critical to effective humoral immunity, the same mechanisms mediate autoimmune and chronic inflammatory pathologies. Accordingly, the importance of understanding the signals and pathways that control adoption of various effector and memory B-cell fates is twofold: it will afford the potential to manipulate response quality in the elimination or control of pathogens, as well as reveal the regulatory circuits that avert errant or overzealous responses that lead to autoimmunity or chronic inflammation.

The recently discovered Age-associated B-cell (ABC) subset exemplifies this dichotomy. Mounting evidence indicates ABCs and their accompanying effector functions are crucial for effective humoral immunity to certain classes of infectious agents, but ABCs are also associated with autoimmunity in both humans and mouse models. These cells display unique phenotypic and functional properties, and make up a continuously increasing proportion of peripheral B cells with advancing age (10–12). Herein, we discuss current understanding of the characteristics and origins of ABCs, contrast them with other major B-cell subsets, and review their roles in both health and disease. Overall, we propose that in all of these contexts, ABCs represent antigen-experi-

enced B cells whose unifying feature is a T-BET driven transcriptional program elicited by antigens that engage endosomal nucleic acid sensors in the context of a promoting cytokine milieu. Finally, we propose a model whereby these features and activation requisites—while enabling effective immunity to infectious agents meeting these criteria—align ABCs with responses to self ligands bearing similar properties, suggesting that responses to these types of ligands must be tightly regulated in order to maintain tolerance to nucleic acid-containing self antigens.

Overview of B-cell development and homeostasis

In order to compare and contrast ABCs with other B-cell pools, the characteristic features of major B-cell subsets are briefly discussed here. Further, as the proportional representation of ABCs progressively increases at the expense of other B-cell pools, a succinct overview of how peripheral B-cell numbers are homeostatically controlled is provided.

Pre-immune B-cell pools

B-cell subsets are defined by surface marker criteria that are correlated with developmental stage, activation status, recirculation properties, and anatomic locale. Table 1 summarizes these criteria, features, and transcriptional regulators for developing and pre-immune B-cell subsets. In healthy adults, B cells are generated continuously from hematopoietic stem cells in the bone marrow (BM), where immunoglobulin (Ig) heavy and light chain gene rearrangements are completed during the pro- and pre-B-cell stages, respectively (13) (reviewed in 14, 15). Commitment to the B lineage involves both the acquisition of a B-cell transcriptional program and the suppression of programs leading to other hematopoietic fates (16). Accordingly, adoption of B lineage fate involves the expression of PAX5 (17–19), EBF1 (20–23), and E2A proteins (24, 25), which establish a transcriptional circuit that not only induces and reinforces B lineage genes but also represses transcription factors driving alternate lineages (26). Upon acquisition of surface IgM, developing B cells enter the immature (IMM) subset. These cells migrate to the periphery via the blood and pass through the so-called transitional (TR) developmental stages before entering the comparatively long-lived mature follicular (FO) or marginal zone (MZ) B-cell pools (27–32). The cues required to adopt the MZ instead of FO B cell fate include tonic BCR signal strength and transcriptional programs driven by Notch2 (33) interactions with Delta-Like-1 (34, 35) reviewed in (36).

Table 1. Features of developing and pre-immune B-cell subsets

Locale	B cell subset	Surface phenotype	Transcriptional regulators	BlyS receptors	Survival cytokine dependence
BM	pro-B	CD19 ⁺ , CD43 ⁺ , CD93 ⁺ , IgM ⁺ , IgD ⁺	PAX5	None	IL7
	pre-B	CD19 ⁺ , CD43 ⁺ , CD93 ⁺ , IgM ⁺ , IgD ⁺	PAX5	None	unknown
	Imm	CD19 ⁺ , CD93 ⁺ , IgM ^{hi} , IgD ^{lo/-}	PAX5	BR3/TACI	unknown
	TR	CD19 ⁺ , CD93 ⁺ , CD43 ⁺ , IgM ^{hi} , IgD ^{lo/-} , CD21 ⁺ , CD23 ⁺	PAX5	TACI/BR3	BlyS
Spleen/ Blood	FO	CD19 ⁺ , CD93 ⁺ , CD43 ⁺ , IgM ^{lo} , IgD ^{hi} , CD21 ^{lo} , CD23 ⁺	PAX5	TACI/BR3	BlyS
Blood, lymph, spleen	MZ	CD19 ⁺ , CD93 ⁺ , CD43 ⁺ , IgM ^{hi} , IgD ^{lo} , CD21 ⁺ , CD23 ^{lo}	Notch targets	TACI/BR3	BlyS
Lymph nodes, Spleen	GC (DZ)	CD19 ⁺ , IgM ⁺ , IgD ⁺ , PNA ⁺ , CD95 ⁺ , CXCR4 ⁺ , CD83 ⁺	BCL6	BR3	BlyS
	GC (LZ)	CD19 ⁺ , IgM ⁺ , IgD ⁺ , CD23 ⁺ , PNA ⁺ , CD95 ⁺ , CXCR4 ⁺ , CD83 ⁺	BCL6	BR3	BlyS
Spleen Blood, BM	SLPC	CD19 ⁺ , B220 ⁺ , IgM ⁺ , IgD ⁺ , CD138 ^{hi}	BLIMP1	TACI/BCMA	APRIL? BlyS? IL6
	LLPC	CD19 ⁺ , B220 ⁺ , IgM ⁺ , IgD ⁺ , CD138 ^{hi}	BLIMP1	BCMA	APRIL?
	Sw	CD19 ⁺ , IgM ⁺ , IgD ⁺ , CD38 ⁺ , PDL2 ⁺ , CD73 ⁺ , CD80 ⁺	unknown	BR3	unknown
	Bmem	CD19 ⁺ , IgM ⁺ , IgD ⁺ , CD38 ⁺ , PDL2 ⁺ , CD73 ⁺ , CD80 ⁺	unknown	BR3	unknown
Spleen Blood, BM	IgM ⁺ Bmem	CD19 ⁺ , IgM ⁺ , IgD ⁺ , CD38 ⁺ , PDL2 ⁺ , CD73 ⁺ , CD80 ⁺	unknown	BR3	unknown
	ABC	CD19 ⁺ , CD93 ⁺ , CD43 ⁺ , IgM ⁺ , IgD ⁺ , CD21 ⁺ , CD23 ⁺ , CD11c ⁺	T-BET	BR3/TACI	unknown

Stringent selection based on BCR specificity occurs during both the IMM and TR stages: only 10% of IMM cells produced survive to exit the marrow, and only 30% of the TR cells thus generated survive to join the FO or MZ pools (28). These profound cell losses reflect both negative and positive selection based on BCR signal strength (37–43), presumably reducing the frequency of polyreactive and self-reactive specificities (38, 40, 44), as well as selecting for cells with optimal subthreshold signal strength (39, 43, 45, 46). Indeed, multiple studies now indicate that the representation of frankly autoreactive B-cell specificities in each pool decreases at each successive differentiation stage (47, 48). Thus, among peripheral B cells, the TR pool is a rich source of autoreactive BCR specificities compared to most other B-cell subsets (49).

The steady-state sizes of pre-immune FO and MZ B-cell pools are controlled by the TNF family member, B Lymphocyte Stimulator (BlyS, a.k.a. BAFF) (50, 51) (reviewed in 52). This cytokine sustains FO and MZ B-cell survival by signals delivered through BlyS receptor 3 (BR3, a.k.a. BAFFR) (53–57). All TR, FO, and MZ B cells express BR3 (58, 59) and compete continuously for BlyS to survive, such that available systemic BlyS determines their lifespan and thus overall FO and MZ pool sizes [(54, 55), reviewed in (60)].

Antigen-experienced B-cell pools

B cells enter functionally distinct subsets following antigen-mediated activation (Table 1). In general, antigen-driven

B-cell responses involve a rapidly generated burst of short-lived plasma cell (SLPC) formation, followed by the expansion of activated FO B cells that initiate germinal centers (GCs) upon receiving CD40 costimulation delivered during cognate interactions with CD4 T cells. B cells within GCs undergo antibody class switch recombination (CSR) to isotypes other than IgM, mediated by the deaminase enzyme AID (61, 62). While these processes are thought to occur most efficiently and frequently in GCs, affinity maturation and CSR in extrafollicular sites have been reported (63, 64). GC B cells are also the targets of AID-mediated somatic hypermutation (SHM) and affinity-based selection, such that mutated clones with higher affinity for the immunizing antigen are selectively preserved. As GCs progress and resolve, they yield B memory (B_{mem}) and long-lived plasma cells (LLPCs). Circulating antibodies have biological half-lives in the range of days to weeks depending on heavy chain isotype; thus, long-term protective immunity must rely on either LLCs that persist indefinitely without seeding from B_{mem} (65–67), or upon the sustained generation of SLPCs from B_{mem} precursors driven by persistent antigen.

To adopt activated and effector fates, pre-immune B cells must integrate instructional cues from a variety of signals, sustain the mutational stress of CSR and SHM, and undergo profound morphological changes to produce antibody. To support these functions, activated B cells undergo significant transcriptional reprogramming based on the cues they receive. Thus, BCR-mediated cell activation in the context of CD40 costimulation induces translation of the transcription

factor BCL6, which is critical for adoption of GC B-cell characteristics (68–70). For example, despite the DNA breaks incurred during CSR and SHM, BCL6-mediated repression of p53 renders GC B cells resistant to apoptosis (71). Differentiation to antibody secretion and the plasma cell fate requires further transcriptional reprogramming. Though initially thought to be required for PC commitment (72), the transcription factor BLIMP1 is necessary for *bona fide* PC differentiation (73). This likely stems from the ability of BLIMP1 to extinguish PAX5 expression, which affords the profound shifts in functional and morphological characteristics associated with PC differentiation (74, 75). In this regard, these cross-competing transcription factors facilitate a gene expression program to direct mature B cells through activation, selection, and terminal effector function. No master regulator of the B_{mem} cell lineage is yet identified; however, T-BET and ROR α are associated with IgG_{2a/c} and IgA respectively, and are critical for the formation and maintenance of some B_{mem} subsets (76).

The homeostatic regulation of antigen experienced B lineage pools is less well understood, and likely involves a more complex set of players than the comprehensive role assumed by BLyS for pre-immune pools. Indeed, the heterogeneity in lifespan and composition of B memory and PC subsets has only begun to be appreciated over the last several years, and will likely expand further. For example, recent conceptual advances challenge the notion that PCs are a monolithic population. Instead, T-cell-independent PCs live longer than previously thought (77); new subset markers have revealed phenotypic heterogeneity and complex turnover kinetics in BM PC pools (78, 79); and some PCs secrete cytokines and antimicrobial agents in addition to antibodies (80). Despite this growing complexity, several factors that govern PC longevity and homeostasis have been identified. LLPC survival depends, at least in part, on BLyS and/or APRIL signaling via the BCMA and/or TACI receptors (81, 82). In this regard, LLPCs are thought to occupy survival niches independent of the primary pool. Homing to and occupation of these longevity-fostering niches is still an active area of research especially given newly discovered subsets and isotype-associated functional differences (83).

Similarly, several B_{mem} subsets have been defined, based on the presence of different isotypes and further markers (Table 1). The immunological role of B_{mem} cells may appear redundant with simultaneous, clonally similar, and elevated antibody titers; however, recent evidence highlights a distinct B_{mem} cell role in response to the pathogenic variants that have escaped the neutralizing capacity of the primary

antibody response (84). Moreover, delineating which B_{mem} subsets rapidly differentiate into PCs or engage in further GC formation upon secondary antigen challenge is an area of intense investigation. Although some evidence suggests that isotype determines B_{mem} cell subset and properties (85, 86), more recent work shows that surface markers such as PD-L2 and CD80 predict functional outcomes irrespective of isotype (87). Furthermore, the maintenance of these pools is independent of BLyS, antigen, or T cells (88–90). *In toto*, these observations indicate that PCs and B_{mem} play non-redundant roles in immunity and occupy distinct homeostatic niches.

The discovery and features of ABCs

Advancing age is accompanied by far-reaching shifts in immune system development and function, including reduced lymphopoiesis (91–94), blunted primary and recall immune responses (95–110), the appearance of autoantibodies (111–116), and increased frequencies of autoimmune and autoinflammatory conditions (117). Accordingly, shifts in the generation and relative representation of lymphocyte subsets have been scrutinized as potential mechanisms underlying these features. Within the T lymphocyte lineage, thymic involution, reduced T lineage specification, and an inversion in the ratio of naive to memory T lymphocytes are established age-associated phenomena (92, 105, 113, 118–124), and more recent studies have linked these shifts to compromised vaccine and disease responses. Within the B lineage, similar reductions in early lineage specification and shifts in the sizes and kinetics of developing B-cell pools have also been appreciated for some time (91, 93, 94, 99, 101, 103, 125–130). However, despite numerous clues indicating changes in the functional attributes of peripheral B cells with age (99, 100, 112, 129, 131–134), whether shifts in naive and antigen experienced B-cell subsets occur with age, as well as the functional consequences of such changes, remained unclear until recently. Within this context, a B-cell subset with unique functional properties was identified and characterized.

ABCs emerge with age and have a unique surface marker phenotype

ABCs were first described in simultaneous reports from Hao et al. and Rubtsov et al., using distinct, but largely complementary phenotypic and functional criteria (10, 11) (reviewed in 12). As their name implies, the splenic ABC

pool emerges in mid-life and continuously enlarges with advancing age. Thus, in healthy adult mice, ABCs are either absent or comprise a negligible proportion of peripheral B cells until about 12 months of age, then increase steadily in both proportion and number (10–12, 135). ABCs are observed in multiple inbred strains and F1 combinations, suggesting they are a common feature of the aging B-cell pool (10). Although the tempo with which ABCs emerge varies considerably among individuals, they generally comprise 30–40% of mature recirculating B cells by 24–30 months of age (135). Moreover, ABCs tend to appear sooner and achieve higher numbers and representation in females (11), although the exact basis for this sex-associated dichotomy is unclear [discussed in (12)].

ABCs display a distinct surface phenotype in mice (Table 1). They are mature B cells, as evidenced by their lack of CD93. Further, while positive for both B220 and CD19, they lack the canonical FO, MZ, or B1 B-cell markers CD23, CD21, and CD43, respectively. Detailed phenotypic, functional, and transcriptome analyses confirmed that ABCs differ from all previously defined B-cell subsets. Although Hao et al. (10) used this combination of features to define the population, Rubtsov et al. (11) employed CD11c as a singularly characteristic marker. Thus, while the B-cell population circumscribed by the criteria in Hao et al. includes the CD11c⁺ cells of Rubtsov et al., it also captures additional cells that lack CD11c. This distinction may underlie some apparent differences in functional attributes discussed below.

Anatomically, ABCs are found in the blood, spleen, and BM, but are rarely observed in lymph nodes (10). While these qualities suggest they are circulating cells, multiple observations suggest their trafficking and homing patterns differ from FO B cells and other recirculating subsets. For example, expression of the follicle homing factor CXCR5 is reduced on ABCs (10), and more recent studies reveal they are enriched at the splenic T:B border, presumably reflecting migratory differences imparted by increased CCR7 expression (136). Based on these observations, it is tempting to speculate that ABCs may be involved in the age-associated alterations in MZ composition previously reported by Birjandi et al. (137). Further, based upon their absence from the lymphatics, coupled with the lack of formal studies probing their circulatory properties, it remains possible that some ABCs are sessile tissue-resident cells. In this regard, further phenotypic analysis of ABCs may reveal their origins and function in health and disease.

ABC activation and survival requirements differ from those of other B-cell subsets

In addition to their distinct phenotype and localization characteristics, ABCs display unique activation requisites and functional attributes. Initial *in vitro* studies revealed that, unlike TR, FO, or MZ B cells, ABCs fail to proliferate after BCR cross-linking, but nonetheless survive (10). In contrast, they exhibit robust proliferative responses to stimulation via TLR9 or TLR7, and despite being refractory to BCR cross-linking alone, concomitant BCR engagement potentiates their proliferative responses to TLR ligands (10, 11). Consistent with these observations, both TLR9 and TLR7 expression are elevated in ABCs (10). There is probably heterogeneity among ABCs in terms of relative responsiveness to TLR7 versus TLR9 ligation; cells identified by the surface marker criteria of Hao et al. show more extensive proliferation to TLR9 than to TLR7 agonists, whereas cells defined by the criteria of Rubtsov et al. display the reverse. Whether this reflects alternative routes of generation, different stages of the ABC differentiation pathway, or other distinctions remains unclear.

ABCs are unusual—and again unlike other B-cell subsets—because they express both BR3 and TACI but are independent of BLyS for their survival (10). Thus, BLyS neutralization *in vivo* spares ABCs, despite eliminating FO and MZ B cells. Because ABCs display the same spectrum of BLyS receptors as FO and MZ B cells, they bind and sequester BLyS equivalently and act as super-competitors for this limiting cytokine, inasmuch as they consume BLyS but do not need it to survive. Accordingly, as ABCs are generated and accumulate, they capture a progressively larger proportion of the BLyS-dependent niche at the expense of the FO compartment. Moreover, this capacity for BLyS-independent survival is similar to what has been reported for B_{mem} cells, although currently defined B_{mem} pools do not express BR3 (138). Given the recent advances in delineating murine B_{mem} subsets (87, 139), exploring potential heterogeneity within the ABC pool may elucidate how ABCs fit into the broader immunological context.

ABCs exhibit a characteristic set of effector attributes

The unique functional attributes of ABCs extend to virtually all B-cell effector mechanisms, including antigen presentation, cytokine secretion, kinetics of PC differentiation, and isotype-switching preferences. Several studies have established that ABCs are effective antigen presenting cells (APCs). Initial findings *in vitro* indicated that ABCs can serve as APCs, in accord with their comparatively high levels of

MHC II, CD80, and CD86 (10). Further, ABC-mediated antigen presentation *in vitro* tends to skew naive CD4 T cells to a T_H17 fate, although this was not exclusive of other CD4 T cell cytokine profiles (10). More recent findings indicate that ABCs are also potent APCs *in vivo* (136). When activated by either TLR7 or TLR9 agonists, ABCs secrete a variety of cytokines, including IFN γ , IL-4, IL-6, and IL-10. Finally, upon activation, ABCs rapidly differentiate to antibody-secreting PCs and tend toward IgG_{2a/c} class switching (136, 140), despite their broad surface IgM and IgD expression (10). Given their propensity to become antibody-secreting cells, ABCs may constitute a population of slowly accumulating B_{mem} cells generated in response to nucleic acid containing antigens. While it is unclear whether isotype or costimulatory molecule expression governs B_{mem}-cell responses (85–87), most ABCs are unswitched and remain uncharacterized for CD80, PD-L2, and CD73 expression. Lastly, ABCs also express CD95 and CD138 based on microarray analysis (11), which are markers associated with the GC and PC cell fates, respectively (see Table 1). Further characterization should distinguish the possibility of distinct ABC subsets or an atypical state of activation. *In toto*, ABC effector mechanisms seem to be largely normal, although skewed toward a type-I response.

ABCs in aging—causes or effects?

Inasmuch as ABCs were first detected by virtue of their progressively increasing representation with age, the question arises as to whether they contribute to age-associated alterations in immune activity and immune responsiveness. This seems likely based on their unique spectrum of activation requirements, antigen presenting capacity, and effector cytokine profile, and several recent findings support this notion.

First, recent studies suggest that ABCs may be instrumental in the decreased B lymphopoiesis associated with age. Ratliff *et al.* (135) assessed the proportion of ABCs in mice at different ages, and found that the degree of B lymphopoiesis depression was proportional to ABC representation with age. Moreover, they showed that ABCs are a potent source of TNF α , and that this cytokine was in part responsible for inhibiting the survival of B lineage precursors *in vitro* and *in vivo*. These observations, coupled with the ability of ABCs to occupy homeostatic space to the detriment of pre-immune FO B cells, are particularly intriguing in light of reports that B lymphopoiesis and robust humoral responses can be rejuvenated in aged individuals by B ablative regimes (141–145).

Second, recent studies have revealed aspects of T_{FH} generation that rely on cognate B-cell interactions differ in young and aged mice. For example, several laboratories have shown that optimal IL-21 and IL-4 production among dendritic cell-primed T_{FH} cells only occurs following antigen presentation by B cells (146–148). However, under *in vivo* immunization conditions that normally foster this T_{FH} profile, aged individuals fail to generate T_{FH} cells with the characteristic upregulation of IL-4 (149). While causal links have not yet been forged, it is tempting to speculate that ABC antigen presentation may foster a T_{FH} cytokine profile that lacks IL-4. Alternatively, T_{FH} cells generated in aged individuals may intrinsically skew toward a cytokine profile lacking IL-4, which might be instrumental in driving ABC generation.

Third, in addition to the decline of B lymphopoiesis and functional T-cell alterations, an increase in anti-dsDNA, anti-nuclear, and other autoantibodies occurs in both mice and humans (111, 114, 150, 151). Indeed, ABC accumulation correlates with serum autoantibody levels, and ABCs isolated from aged mice and stimulated with a TLR7 agonist produced anti-chromatin antibodies (11). While not themselves PCs, ABCs could constitute a pool of B_{mem} cells that feed a population of autoantibody secreting PCs. Thus, while ABC accumulation has a number of correlative associations with B-cell development, altered T-cell functionality, and autoantibody production, causality is yet to be established.

ABCs in health and disease

The differentiative routes to an ABC phenotype, the signals prompting this program, and the progenitor-successor relationships involved, have been the subjects of ongoing investigations for several years. Early studies ruled out the possibility that ABCs represent the product of aberrant or skewed B-cell development in the aged BM, inasmuch as ABCs do not reappear during autoreconstitution after B-cell depletion by sublethal irradiation or other regimes (10). Instead, accumulating evidence suggests that ABCs are antigen-experienced B cells arising from activation with nucleic acid-containing antigens in the context of a promoting cytokine milieu. Further, they can likely arise from most pre-immune peripheral B-cell subsets, including the FO and TR pools. The antigens that engender an ABC-like response are intracellular pathogens such as viruses. In this setting, viruses provide three key signals that foster ABC identity: BCR ligation, nucleic acid sensing TLR engagement, and an inflammatory cytokine milieu. However, B cells with some degree of specificity for nucleic acid

containing self-ligands may also receive the requisite three signals leading to ABC characteristics (Fig. 1).

ABCs are products of a T-BET driven transcriptional program

Transcription factors guide and reinforce cell fate decisions for proper biological functions. In accord with this general developmental principal, early transcriptional profiling revealed that ABCs express the transcription factor T-Box Expressed in T cells (T-BET) encoded by the *Tbx21* gene (11). Initially, Glimcher's group described the role of T-BET in CD4 helper T cells as a regulator of IFN γ production and T_H1 cell fate (152). Despite this moniker, several other cell types including CD8 T cells, Natural Killer cells, and dendritic cells express and require T-BET for their specific immunological function (reviewed in 153). In the B lineage, T-BET expression fosters switching to IgG_{2a/c} (76, 154, 155) and is necessary for controlling viral titers (140). Indeed, early studies across multiple mouse strains infected with both RNA and DNA viruses representative of 11 different genera elicited IgG_{2a/c} isotypes, which have subsequently been shown to be protective (156, 157).

Given T-BET's association with humoral autoimmunity and role in protective anti-viral humoral responses, the factors responsible for its upregulation are of particular interest. While the external cues driving B-cell T-BET expression were initially reported as concomitant IL-12 and IL-18 stimulation (152), subsequent studies have also demonstrated that IFN γ shares this capacity (158, 159). Under most circumstances,

these cytokine signals are derived from T_{FH} cells. In order for both GC B cells and T_{FH} cells to fully differentiate, they must engage in intimate and reciprocal interactions beyond the initial T-cell priming event with a dendritic cell (146). T_{FH} cells are transcriptionally and anatomically distinct from other T-helper lineages (160), yet they still harbor the capacity to make IFN γ and IL-4 against viruses and helminthes, respectively (161–163). In addition to the effects of the hallmark cytokine of the T_{FH} lineage, IL-21 (164, 165), how all of these cytokine signals are integrated to instruct appropriate isotype and B-cell fate decisions remains unclear. Thus, the induction of T-BET in the B lineage relies on particular classes of antigenic stimuli—including those provided by DNA and RNA viral infections—in the context of inflammatory cytokines. In toto, the emergence, functional attributes, and T-BET driven nature of ABCs suggest they may reflect the sum of accumulated responses to antigens fulfilling these activation criteria over the lifespan of the host organism. Accordingly, while ABCs will increase steadily within an individual, the rate will vary depending on exposures to appropriate stimulatory cues, and will thus vary substantially across a population. This strikes a cautionary note for assessments of ABC accumulation in humans, in as much as they are both generically diverse and are heterogeneous with respect to antigen exposure history.

ABCs are generated in response to intracellular infections

Early adoptive transfer studies revealed that FO B cells give rise to ABCs. In these studies, CFSE labeled FO B cells were

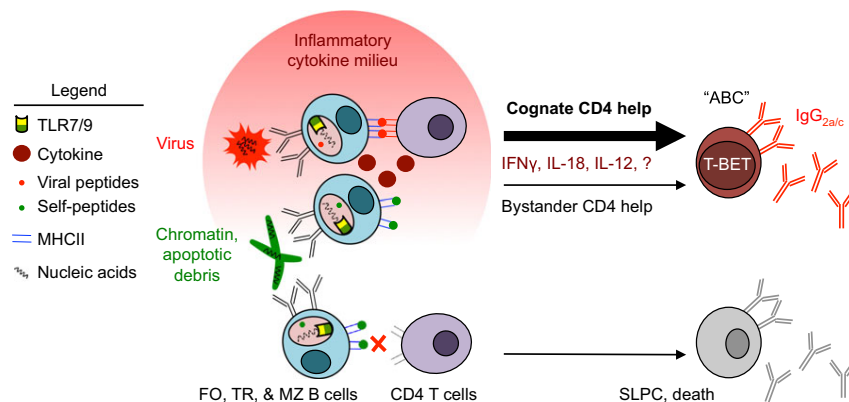


Fig. 1. Both beneficial and pathogenic Age-associated B cells (ABCs) arise via a common triad of signals. Pre-immune FO, MZ, and/or TR B cells bind, internalize, and traffic antigens to endocytic compartments via the BCR. Pathogen degradation leads to viral nucleic acids engaging TLR7 or TLR9. Processing and loading of viral peptides onto MHCII molecules results in cognate CD4 T-cell interactions and T-cell-derived inflammatory cytokine secretion. Currently, IFN-gamma, IL-18, or IL-12 are known to serve this role, but additional, yet to be interrogated, cytokines (depicted as "?") may also positively or negatively regulate ABC fate. The combination of these signals leads to T-BET⁺ memory and effectors, and to IgG_{2a/c} isotype switching. Normally, B cells that internalize nucleic acid containing self-antigens fail to survive because they lack the cognate T-cell interactions and appropriate cytokine signals to further differentiate and survive. However, inadvertent or aberrant receipt of these signals can afford survival of these self-reactive cell and enable their recruitment into a long-lived memory and effector ABC pool.

transferred to congenic hosts and harvested 30 days afterward, revealing that transferred cells which had extensively proliferated had acquired the ABC phenotype (10). This observation linked the generation of ABCs with extensive division, although the stimulating conditions driving the phenotype were unclear, and further functional characterization of these recently formed ABCs was hampered by the small cell numbers recovered in such experiments. Nonetheless, these findings suggest that ABCs are likely an antigen-experienced pool. Since B-cell responses against viruses result in a protective, T-BET-dependent IgG_{2a/c} response, Marrack's group hypothesized that a viral infection would drive T-BET *in vivo*. Indeed, mice infected with gamma herpes virus, vaccinia, or LCMV all produced T-BET⁺CD11c⁺ B cells (140). Of the TLR agonists used, the authors found TLR7 stimulation to be the most effective at driving T-BET expression in the context of BCR and IFN γ stimulation. Lastly, B-cell intrinsic T-BET expression played a non-redundant role in controlling viral and anti-viral IgG_{2a/c} production. These findings have been extended to human studies where a subset of B cells in HIV-viremic individuals also display an ABC surface phenotype (166), but are yet to be fully characterized for T-BET and CD11c. *In toto*, these data support the notion that ABCs are indeed antigen experience cells arising from immune responses characterized by nucleic acid-containing antigens in the context of pro-inflammatory cytokines. Whether other inflammatory cytokines harbor the capacity to induce an ABC-like phenotype and effector function remains unclear. Nonetheless, given the similar nature of the antigens, nucleic acid-containing self-antigens, like viruses, may drive a similar ABC-like program.

ABCs are associated with humoral autoimmune and autoinflammatory diseases

Beyond their initial identification in aged mice, ABCs prematurely accumulate in a variety of autoimmune-prone mouse strains. A considerable ABC population accumulates as early as 6 months of age in mice lacking the tyrosine kinase, Mer, as well as in NZB/WF₁ animals (11). In accord with these findings, ABCs constitute a higher proportion of total B cells in female RA patients (11). Moreover, before their detailed characterization in mice, ABC-like cells were also observed in common variable immunodeficiency (167) and Sjögren's syndrome patients (168). It is unclear if the CD21^{-/lo} cells in these individuals constituted a *bona fide* ABC population, since they were not characterized for CD11c, T-BET, or other

subsequently identified ABC makers. In contrast, a more recent investigation of an early-onset Evans syndrome patient exhibited premature senescence and an increased number of CD11c⁺T-BET⁺ B cells that correlated with antinuclear antibodies (169). Interestingly, duplicating the tripeptidyl peptidase II (TPP2) frameshift mutation in mice recapitulated both the disease and ABC phenotype. Thus, ABCs are implicated in both human autoimmune syndromes and in murine models of humoral autoimmunity. These findings prompt the question of how the dichotomous roles of ABCs—providing beneficial and appropriate effector functions during intracellular infection but also being associated with humoral autoimmunity—can be mechanistically reconciled.

A unique signaling triad links appropriate and pathogenic ABC generation

ABCs in aged, virally infected, and autoimmune-prone mice and humans share a common triumvirate of stimuli for their origin: BCR signals, endosomal nucleic acid sensor ligation, and a type-I inflammatory cytokine milieu. This commonality is likely the link that connects beneficial and pathogenic ABC formation. In particular, the role of endosomal nucleic acid sensors driven by BCR-acquired antigens may prove the most important feature. Over the past decade the contribution of nucleic acid-sensing PRRs, particularly TLR7 and TLR9, to the etiology of autoimmune disease have come to the forefront. Beginning with the seminal observation that BCR delivered nucleic acid ligands have distinct survival and proliferative potentials for B cells (170), it has become increasingly apparent that TLR7 and TLR9 both promote and regulate the progression of humoral autoimmunity. To test this idea, Shlomchik's group bred SLE-prone mice to either TLR7 or TLR9-deficient mice (171, 172). Because extra *Tlr7* gene copies result in an SLE-like disease (173), it was expected that both TLR7 and TLR9 deficiency would ameliorate disease in autoimmune-prone mice. While TLR7 deficiency yielded the expected result, TLR9 deficiency unexpectedly exacerbated disease (171, 174). This suggests not only a regulatory role for TLR9 but also differential disease outcomes based on whether autoreactive B cells can sense RNA or DNA moieties. These relationships are further complicated by the fact that TLR9 deficiency in other models of autoimmunity ameliorates disease (175). Though the mechanism by which TLR9 limits disease remains unclear, recent findings suggest a role for type-I interferon signaling in disease etiology (176). Whether ABC formation occurs in these

models of autoimmunity and whether TLR7 and TLR9 modulate ABC accumulation is yet to be established. Thus, the signals from the BCR, nucleic acid sensing TLRs, and inflammatory cytokines—while involved in appropriate ABC-mediated immunity—are exactly those that, when dysregulated, foster humoral autoimmunity.

These ideas lead to the model schematized in Fig. 1. In general, any nucleic acid-containing antigen harbors the capacity to drive an ABC phenotype. However, growing evidence suggests that T-cell derived pro-inflammatory cytokine signals, as well as cognate help, may be required to engender a long-lived ABC fate. In this regard, an activated B-cell presenting viral peptides will efficiently receive all three signals required for recruitment into long-lived effector subsets. In contrast, autoreactive B-cells binding nucleic acid-containing self molecules—such as apoptotic debris or other sources—while receiving BCR and TLR signals, should fail to receive further cues for GC formation and survival, resulting in short-lived responses or death. However, inadvertent or aberrant recruitment of such short-lived autoreactive cells into long-lived effectors would thwart this peripheral tolerance system, and yield sustained autoantibody and auto inflammation driven by IgG_{2a/c} production. Multiple routes to such dysregulation could be envisioned,

including overabundance of autoantigen or deficiencies in the molecules that mediate these regulatory circuits.

Perspective

ABCs impact a broad spectrum of immunological phenomena, including protective immunity to some pathogen classes, immunosenescence, and autoimmunity. In mice, ABCs constitute an accumulating population of B cells with unique surface phenotype, signaling properties, and effector status. While initially described in aged mice, phenotypically and transcriptionally identical cells are observed in humoral autoimmunity and immune responses against intracellular pathogens in both mice and humans. These observations lead us to propose that ABCs are a memory B cell population, and that the signals and interactions that yield ABCs in both normal and autoreactive humoral responses are comparable; these consist of concomitant signals from the BCR, nucleic acid sensing TLRs, and inflammatory cytokines. Thus, understanding how responses to endogenous versus exogenous nucleic acid-bearing ligands are regulated in order to maintain tolerance yet allow appropriate responses to pathogens should yield insights relevant to both protective and autospecific humoral immunity.

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